

Mesoscale Simulation and cryo-TEM of Nanoscale Drug Delivery Systems

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This work sets out to study the effect of hydrophobic molecules on the morphology of aqueous solutions of amphiphilic block copolymer, which has potential drug delivery applications. The effect is studied both experimentally and by using simulations. Using cryogenic TEM observations, micelles can clearly be visualised and their core size measured. While pure polymer solutions form into spherical micelles with a narrow size distribution, addition of small amounts of hydrophobic drug molecules leads to distortions in shape, a wider size distribution, and larger average core diameter. Simulations are based on a mesoscale dynamic density functional method with Gaussian chain Hamiltonian and mean-field interactions, as implemented in the MesoDyn code. With parameters for the amphiphilic system established in earlier work, and mean-field interactions for the drug molecule derived from structure–property relationships, we obtain good agreement with the TEM observations for the effect of the hydrophobic molecules on the morphology. The simulations clearly show how increasing drug concentration leads to an increase in micelle size, a wider distribution and more elongated rather than spherical micelles.

Keywords: Block copolymers; Micelles; Drug delivery; cryo-TEM; MesoDyn

INTRODUCTION

Block copolymers consisting of alternative blocks of poly(ethylene-oxide) (PEO) and poly(propylene-oxide) (PPO) can be used as nano-size containers for hydrophobic drugs due to their special solution behaviour. Above a certain critical concentration or critical temperature, these amphiphilic molecules

aggregate to form micelles, with the core made up of the hydrophobic part of the chain and the corona consisting of the hydrophilic part of the chain. Numerous research groups have carried out indirect studies on the micellar morphology of this class of polymers but very few have physically observed these micelles. By using direct visualisation techniques, such as cryogenic transmission electron microscopy (cryo-TEM), we can directly observe the morphology of triblock polyethylene oxide–polypropylene oxide without having to assume models to fit the experimental data. Direct visualisation allows one to study the structure of micelles and hence gain a better understanding of the effect of additives such as hydrophobic drugs.

In parallel to the experimental work, we have studied the morphology of aqueous solutions of block copolymer [1] using the MesoDyn program from Accelrys Inc. MesoDyn is based on dynamic mean-field density functional theory. The phase separation dynamics is described by Langevin equations for polymer diffusion [2]. One important advantage of this method is that there is no *a priori* assumption on the phases, and that the kinetics of phase formation, which is very difficult to observe experimentally, can be studied.

In the first part of this work, there will be a discussion of the experimental evidence regarding the morphology of the micelles and the effect of dissolved hydrophobic drug molecules on the structure. In the subsequent part, we will show if the atomistic detail of the polymers mapped onto the coarse-grained description used in MesoDyn can reproduce these results by simulation.

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EXPERIMENTAL METHOD

Materials

The copolymer used in this study is an (ethylene oxide)(propylene oxide)(ethylene oxide) or (EO)_m(PO)_n(EO)_m triblock copolymer of composition: $m = 99$, $n = 65$. This copolymer is designated commercially as F127. The polymer is obtained from Fluka and has been used without any further purification. In addition to the polymer mentioned, a drug commercially known as Haloperidol (C₂₁H₂₃ClFNO₂) is used as the hydrophobic molecule to study the effect of these molecules on the morphology of the micelles. The crystalline drug is also obtained from Fluka and used without further purification.

The copolymer is dissolved by weight at 5°C in distilled water and incubated at above micellisation temperature for 24 h before being used for experiments described in the next section.

For the samples containing the hydrophobic drug molecule, in order to get a better mixing of the molecules with the polymer, both the polymer and the drug are first dissolved in chloroform (trichloromethane). Then the mixture is added to water at about 60°C in a BUCHI RE III Rotavapor with a pressure of 0.7–0.5 bar. The mixture is constantly stirred until all the chloroform is removed. The elevated temperature prevents the recrystallisation of the drug and also aids in the removal of chloroform. The solution is then incubated at about 30°C for 24 h before being used in the experiment described next.

Electron Cryo-microscopy

Samples for observation under a transmission electron microscope (TEM) have to be very thin films consisting preferably of a single layer of micelles, and free from ice crystals. In order to obtain such samples, solutions of various concentrations of each sample were vitrified according to the following procedure. A solution of about 2 μl was applied to a holey carbon grid, which was mounted on a controlled environment freezing apparatus maintained at 40°C and very high humidity. After about 30 s, the grid was blotted from the carbon side for about 5–10 s with a double layer of filter paper (Whatman no. 1) before plunging into liquid ethane. The different concentrations of polymer require different blotting time to achieve the right thickness. One possible effect of this is the removal of different amounts of water from different specimens, resulting in an uncontrolled change in morphology. However, micrographs of samples made from the same solution at different blotting times showed no changes in microstructure. Hence, we can assume that

the timescales for relaxation are much longer than the blotting time of the samples.

The vitrified sample was then transferred to a Gatan cold holder, which keeps the sample at liquid nitrogen temperature (~170°C). Image of the micelles in amorphous ice over holes was then recorded under low dose conditions from an untilted sample at a magnification of 60,000X on a Philips CM12 or JEOL 2000FX electron microscope fitted with a tungsten filament. For the micelles to be visible and stable in the microscope, the final film thickness has to be exactly right and this proved to be very difficult.

Image Analysis of Electron Micrographs

Image processing is commonly used for various aspects of electron microscopy, such as three dimensional reconstruction from two dimensional images, extraction of signal from noisy images and also feature quantification [3]. There are various commercial software packages available for these tasks. We have used the *Semper* software to process and analyse the micrographs.

The analysis is performed on a scan of the micrograph of a particular sample. After Fourier transformation of the image data, the significant feature in the image is further enhanced.

Experimental Results and Discussions

The following two samples were prepared as described above: 10% wt. F127 and 10% wt. F127 with 0.2% wt. Haloperidol.

Micellisation Morphology

Micellisation morphology has been a subject of immense interest because of its numerous potential applications. Many of these crucially depend on the details of morphology, such as in the case of latex formation, or in their use as nanoscale containers for hydrophobic drugs. In this section, we develop our earlier work [4], in which we demonstrated that direct visualisation is possible on a material that has blocks with very low electronic contrast as in PEO-PPO triblock copolymers. In previous studies by Mortensen and Talmon [5], the contrast for the samples was very poor.

Using DSC measurements, we can put an upper bound on the micellisation transition, which provides us with a “safe” temperature at which to prepare samples for the cryo-TEM observation of micelles. A typical image of the micelles in one of the holes in the carbon film is shown in a previous paper [4].

The micro-phase separated structures are clearly visualised due to the contrast achieved by

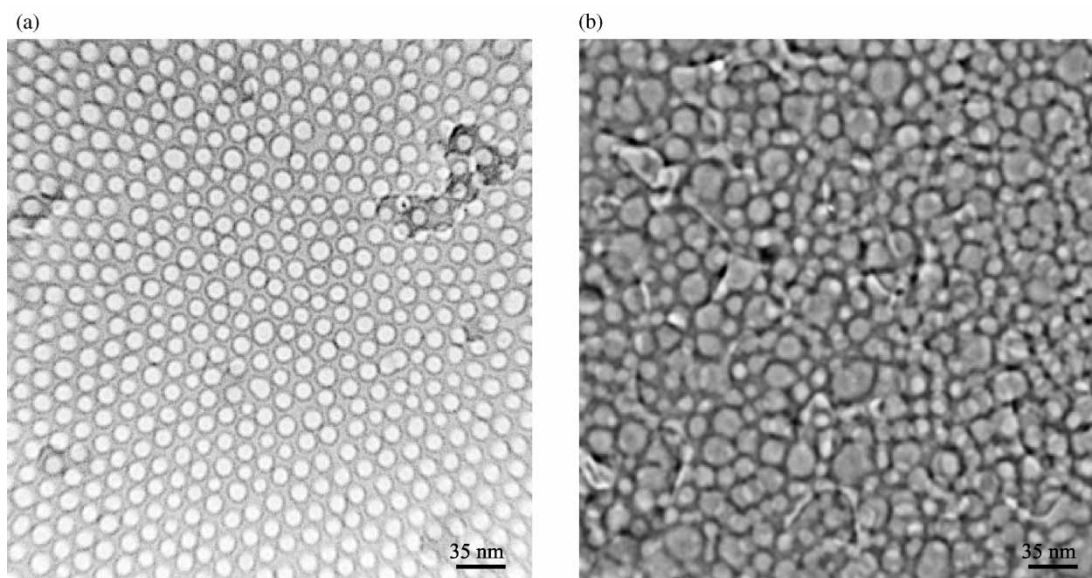


FIGURE 1 Effect of the hydrophobic molecule on the morphology of the micelles. (a) 10% wt. F127, (b) 10% wt. F127 with 0.2% wt. Haloperidol.

underfocussing. Individual micelles can clearly be distinguished as the micrograph of F127 in Fig. 1(a) shows. They are generally spherical in shape and exhibit a core–corona structure. The bright rings, or sometimes half rings, seen between the core and the corona of some micelles are Fresnel fringes. Underfocussing produces a dark Fresnel fringe just outside the “micelle” and a bright Fresnel fringe just inside the “micelle” enhancing the contrast between the “micelles” and the solution. It is difficult to tell whether the “micelle” seen in the TEM image is indeed a micelle in the typical sense (i.e. the core and the corona) or just the core from the micrograph. Since the corona contains both PEO and associated water molecules, the electron density is expected to be closer to that of water. Hence, the bright “micelle” that was discussed earlier most probably includes the core and part of the dense PEO layer around the core of the micelle [4]. This bright region will be referred to as core of the micelles. The core appears to be lighter because it scatters fewer electrons to high angles. This is consistent with the core of the micelle containing PPO (scatters less) and the solution containing PEO. In addition, there is some evidence of a transition zone between the core and the interface in the form of a shoulder inside the bright peak in Fig. 2.

Effect of Incorporated Hydrophobe

The core of the micelle is made up of the hydrophobic middle block of the chains (PPO). This hydrophobic core is the perfect microcapsule for hydrophobic molecules to reside in. The effect of the hydrophobic small molecule on the micellar morphology can be seen from the micrograph in Fig. 1(b). There are several changes in

the morphology. The micelle size distribution increases as some of the micelles increase dramatically in size, while others are similar in size or even slightly smaller than in the pure F127 solution. Furthermore, there are some micelles which appear less spherical than those observed in the sample without the hydrophobic molecule. This may be caused by recrystallised drug as this generally leads to a less equiaxed morphology.

Figure 2 shows line scans of micelles with and without the haloperidol. For the case with haloperidol, a micelle from the population of enlarged, but approximately spherical aggregates was chosen. The core diameter of the “haloperidol filled” micelle is substantially larger, in fact about twice the size of the unfilled micelles. This is in line with visual inspection of the micrographs in Fig. 1. Such a substantial size increase cannot be the result of the inclusion of such a small amount of hydrophobic molecules in the core of the micelles alone (see also our discussion of the simulation results below). Possible reasons of the large size are a very uneven distribution of the haloperidol amongst the micelles, crystallisation, and a wider distribution of the haloperidol into the corona combined with the fact that the haloperidol will show up strongly in the TEM due to the presence of chlorine and fluorine.

MESOSCALE SIMULATIONS

As discussed in a previous paper [1], Dynamic Mean-Field Density Functional Theory, as implemented in the MesoDyn software, can be used to simulate solution behaviour of specific chemical species. To specify the chemical nature of

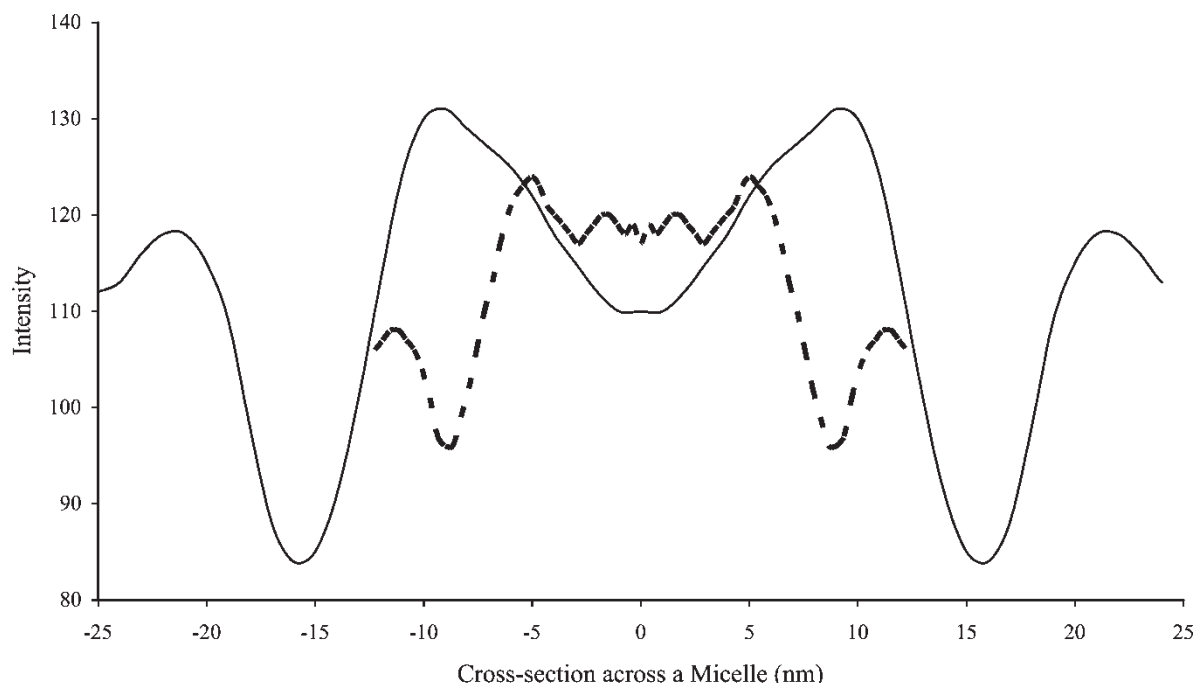


FIGURE 2 Cross-section intensity profile of micelles observed by cryo-TEM, as shown by (---) for without haloperidol and by (—) for with haloperidol.

the system, there are two sets of parameters which have to be defined. The two parameter sets are related to the Gaussian chain architecture and the interaction energies of the various components. For the purpose of evaluating the density functionals, we can replace the real molecular chains with a Gaussian chain that has the same response functions as the “real” molecular chain and specify the interaction between each species. The first parameter, the Gaussian chain architecture, depends on the degree of coarsening of the original system. For each system, there is a specific characteristic length, similar to that of the Kuhn length, which translates to the Gaussian chain bond length of chains in MesoDyn. In the case of PEO-PPO-PEO triblock copolymer, this single bond length will represent a certain number of monomers of PEO and a different number of PPO. Different procedures for determining the bond length of the Pluronics systems using atomistic simulations have been described in previous papers [1,2]. They typically involve methods to generate ensembles of chains, and an analysis such as structure factors for the Gaussian chain fitting. The method used in our work is the RIS Metropolis Monte Carlo (RMMC) in the Cerius [2] software from Accelrys. The resulting Gaussian chain architecture for F127 is $A_{24} B_{20} A_{24}$, and the bond length is 1.1 nm. To define the size of the hydrophobic molecule, an atomistic simulation of haloperidol was carried out again using the RMMC method. The average end-to-end distance taken over the 1000 minimised conformations of the chain (Fig. 3) is approximately 1.14 nm, very similar

to the length of a polymer bead. It is, hence, a good approximation to represent the hydrophobic molecule with a single bead.

The second parameter is the mean-field interaction energy between the different chemical components, which also captures the hydrophilicity and hydrophobicity of the components.

The interaction parameters concerning EO, PO and water were retained from previous work [2] while those between EO, PO and haloperidol, respectively, were calculated using the Group Contribution Method [6]. The parameter between haloperidol and water was obtained [7] by fitting the experimental solubility data to obtain regression equations, which gave a reasonable prediction of the solubility of haloperidol in the solvent. The interaction parameter values are given in Table I.

SIMULATION RESULTS AND DISCUSSIONS

For most of our simulations, instead of F127, we used P85: $PEO_{40}PPO_{26}PEO_{40}$ triblock copolymer, represented by a Gaussian chain $A_6 B_{12} A_6$ with beads similar in size as for F127. As shown in the previous paper, the core radii obtained for both F127 and P85 are comparable, but due to the shorter chain length, the P85 system is computationally much less demanding. Since both chains are made up of similar components, the interaction with the hydrophobic drugs should be the same. The parameters used for this simulation are provided in the Appendix.

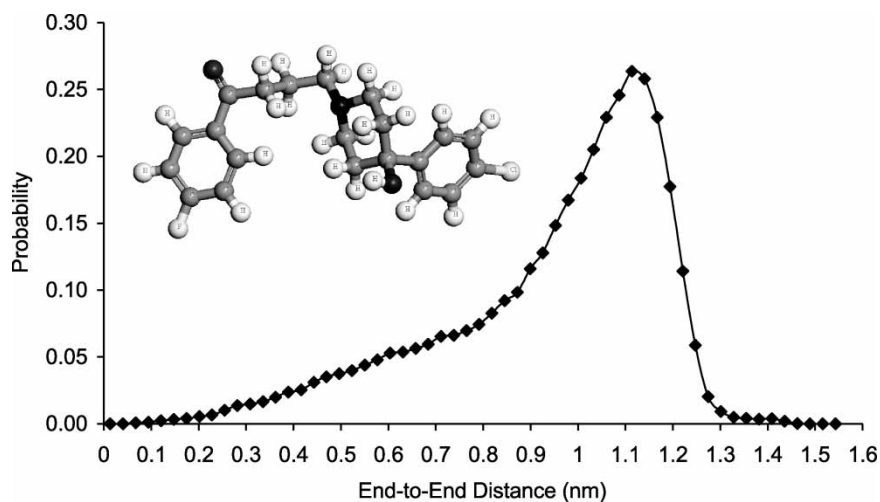


FIGURE 3 End-to-end distance for 1000 conformations of haloperidol (structure shown in the inset), as obtained from RMMC simulations.

TABLE I Interaction parameters of haloperidol

Interacting molecules	χ
Haloperidol–Water	4.88
Haloperidol–PEO	2.070
Haloperidol–PPO	1.125

Effect of Incorporated Hydrophobe

Hydrophobic molecules in a micellar solution tend to “reside” in the core of the micelle away from the water. Hence, a small number of hydrophobic molecules are expected to enlarge the micelle core radius slightly. In order to study this effect, several concentrations were simulated ranging from 0.1 to 4% by vol. of the haloperidol molecule in 24% vol. aqueous solution of P85 at 298 K.

The simulation was run for 40,000 time steps and equilibrium is reached as seen from the order

parameter plot shown in Fig. 4. From about 0.1 to about 3% vol. of hydrophobe, the micelles formed are spherical as seen in the isodensity plot shown in Fig. 5(a). As the concentration is increased further the micelles tend to become more rod-like (the isodensity plot of the 4% vol. of hydrophobe case in Fig. 5(b)).

We carried out an analysis of the micellar aggregates in terms of their size distribution, number density, core volume and average radius. Here, the micelle aggregate is defined as being bounded by the isodensity surface of the PO component at half the maximum value. Figure 6 shows the distribution of micelle aggregate volumes for the cases of 0.1 and 2% concentration of haloperidol. First of all, we note that the average size increases with concentration, but also that the micelles are not uniform in size, and that the difference between largest and smallest micelles, i.e. the width of

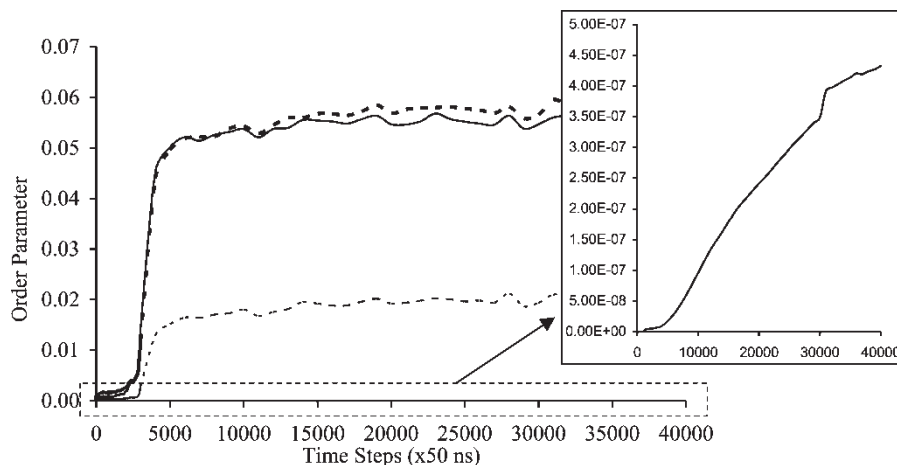


FIGURE 4 Density order parameter for evolution during the simulation. Inset shows the order parameter of haloperidol, which has a much smaller value because of the small concentration of molecules present. PEO (---), PPO (—), Water (-----), Haloperidol (—).

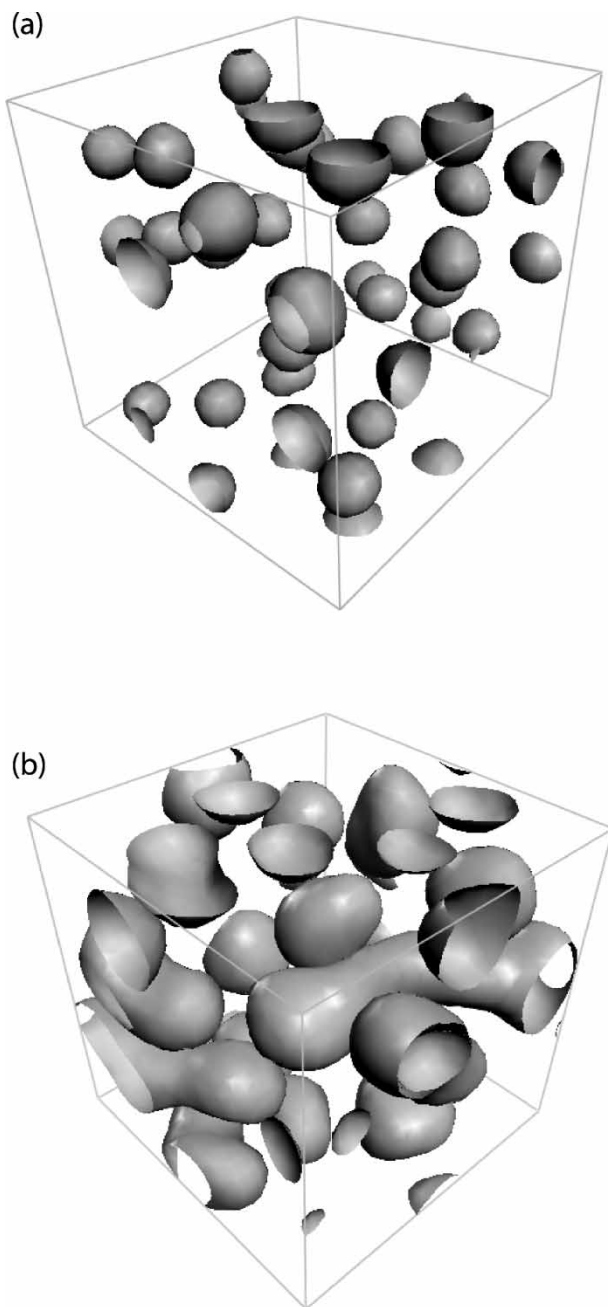


FIGURE 5 Isodensity surfaces of the PPO component at half the maximum concentration, taken here as a delineation of the micelle core. (a) 24% vol. PEO₂₆PPO₄₀PEO₂₆ and 0.1% vol. haloperidol, (b) 24% vol. PEO₂₆PPO₄₀PEO₂₆ and 4% vol. haloperidol.

the distribution, also increases with increasing haloperidol volume fraction. The difference between the radii of largest and smallest micelles is about 0.8 nm for $c = 0.1\%$, 1.2 nm for $c = 2\%$ and 2.3 nm for $c = 4\%$. Figure 7(a) shows the average radii, indicating also the distributions with error bars.

The increase in size can be calculated arithmetically. The average radius of the micelles in a system without any haloperidol at 20,000 time steps is about 2.8 nm, and the average radius of micelles in a system with about 2% vol. haloperidol is about 3.1 nm. Based on the size of

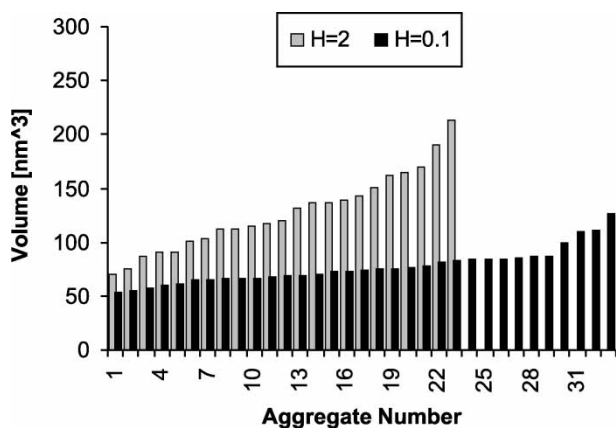


FIGURE 6 Aggregate analysis for haloperidol concentrations 0.1 and 2%, showing the volume of each of the micelles in the simulation box. Note the decreasing number of micelles and increasing size for the higher concentration.

the simulation cube, if there are 2% vol. of hydrophobic molecules present, the total amount of hydrophobe will be about 737.7 nm^3 . Since there are about 22 micelles, assuming equal distribution of the hydrophobes, the increase in the size will be about $3 \times 10^{-1} \text{ nm}$ in radius. This is very close to what we observed. Hence, we can be confident that the simulated size is indeed reasonable.

The aggregate analysis also shows that the number of micelles actually decreases with increasing haloperidol concentration (Fig. 7(b)). Since the concentration of Pluronic is kept constant, this means that the aggregation number of the micelles has increased: not too surprising if one considers the fact that the haloperidol helps to increase the micelle core size, providing more surface area for the hydrophilic tails of the Pluronic molecules to occupy.

A more detailed analysis of the haloperidol distribution across the micelle illustrates this picture. With a very small haloperidol addition, the hydrophobic molecule sits in the core and the interface between the core and the corona regions as shown in Fig. 8(a). With an increase in the hydrophobic molecules, there is an increase in the repulsion between the core and water, and hence the poor

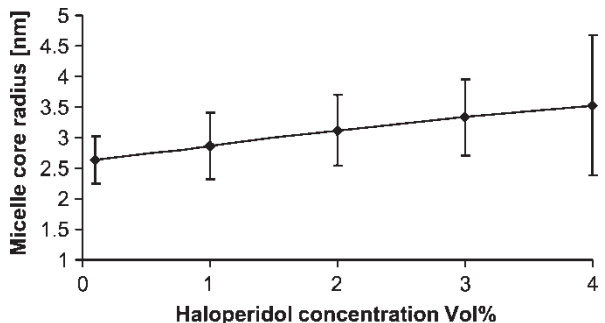


FIGURE 7 Effect of haloperidol concentration on the average radius and size distribution of the micelle cores.

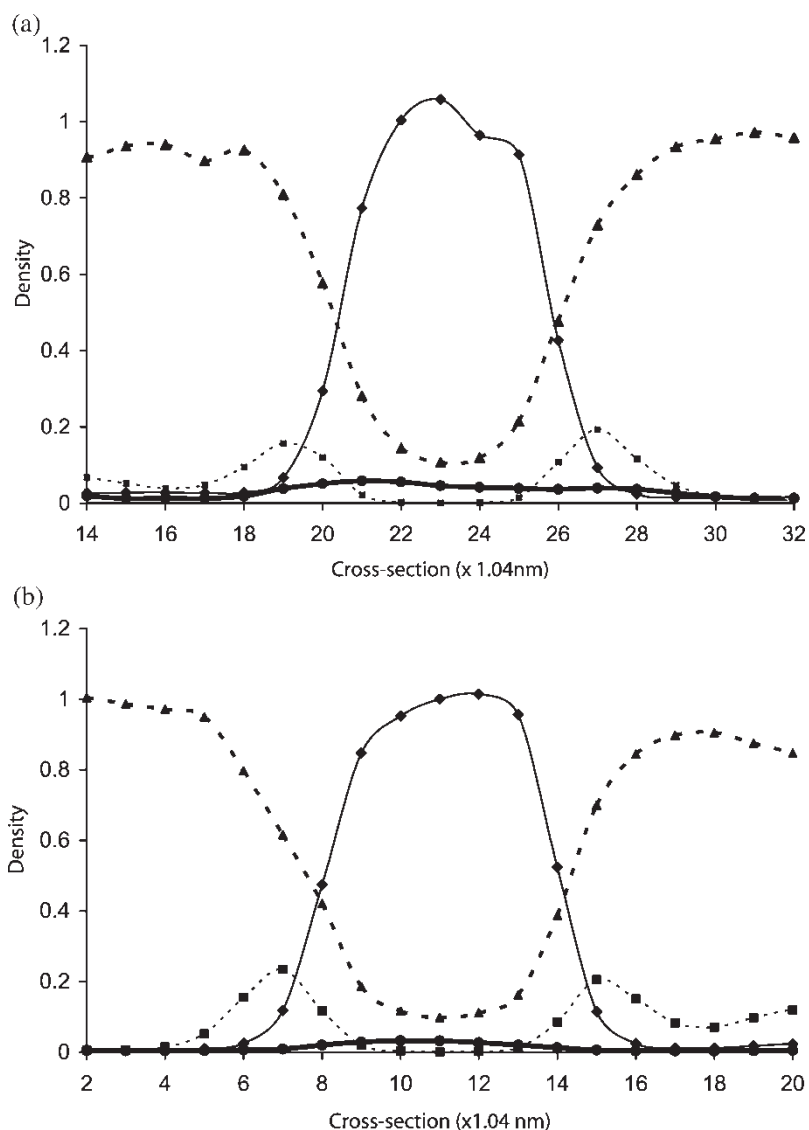


FIGURE 8 Density profile of (a) 24% wt. PEO₂₆PPO₄₀PEO₂₆ with 0.1% wt. of haloperidol, and (b) 24% wt. PEO₂₆PPO₄₀PEO₂₆ with 1% wt. of haloperidol. PEO (—■—), PPO (—◆—), Haloperidol (—●—), Water (—▲—).

solvent effect is more prominent. Malmsten and Lindman [8] made exactly the same observation with the addition of hydrocarbon. The result of this is the more rod-like structure. By comparing the density distribution of the system with higher concentration of hydrophobic drugs, the hydrophobic molecules tend to move away from the core and corona interface and aggregate in the centre of the core as shown in Fig. 8(b). These observations confirm that with the addition of a hydrophobe such as haloperidol, the poor solvent effect is increased and hence the system is effectively pushed to an excluded volume condition. Morphologies that are normally formed at high concentration and temperature are formed at a lower concentration and temperature.

The tendency towards a rod-like morphology at higher haloperidol concentration (Fig. 5(b)), which otherwise is formed at much higher concentrations

of P85 solutions, is completely consistent with the above findings. As the addition of haloperidol also increases the aggregation number, eventually a rod-like micelle becomes the lower energy structure.

Finally, a comparison of the effect of hydrophobic molecules in both experimental and simulation studies is due. In the cryo-TEM, we observed that a small addition of haloperidol results in a substantial increase in the size of some micelles. This is in contrast to the results obtained in simulation. The reason for this difference may be due to recrystallisation of the haloperidol during the experimental sample preparation. As this generally results in the preferential growth of some larger nuclei, there is a large size distribution for the micelles. Some micelles will remain unchanged while the sizes of other micelles increase tremendously.

CONCLUSIONS

It is known that hydrophobic molecules can be dissolved in the micellar solution. In this work, the effect on the morphology has been investigated in detail both by cryogenic TEM and simulations. From the TEM images, it is obvious that the core size of the micelles increases significantly with the presence of a very small amount ($\sim 0.2\%$ wt.) of hydrophobic molecules, haloperidol ($C_{21}H_{23}ClFNO_2$). Some of the micelles also seem to be less spherical than before the addition of the hydrophobe. This could be due to the nature of the drug, which crystallises into long crystals when there is a substantial local concentration of the small molecules.

MesoDyn simulations have been performed with parameter values derived previously for Pluronic (ethylene oxide)(propylene oxide)(ethylene oxide) triblock copolymers, and additional parameters determined here for the interactions with haloperidol.

The simulations show similar effects of the hydrophobic molecules on the morphology of the micelles, in particular the increase in micellar size with the addition of hydrophobic molecules. They also show an increase in the width of size distribution, and aggregation number. At higher concentration ($\sim 4\%$ vol.) than those tested experimentally, a more rod-like phase is formed. This morphology is similar to that seen at higher concentrations or temperatures for pure solutions. The reason for this is that the addition of the hydrophobic drug, which tends to aggregate in the core of the micelles, effectively increases the repulsive energy between the chains and the water. This results in a condition similar to a poor solvent quality induced by either increasing the temperature or a higher concentration of polymer.

Acknowledgements

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APPENDIX—INPUT PARAMETERS FOR THE SIMULATION RUNS

PEO₂₆PPO₄₀PEO₂₆—Pluronic™ P85

Bead—PEO

Name A
Diffusion_coefficient 1.000E-07

Bondlength 1.20
Volume 0.30

Bead—PPO

Name B
Diffusion_coefficient 1.000E-07
Bondlength 1.20
Volume 0.30

Bead—Water

Name W
Diffusion_coefficient 1.000E-07
Bondlength 1.20
Volume 0.30

Interaction Energies

Interaction—epsilon A A 0.000
Interaction—epsilon B A 7.300
Interaction—epsilon B B 0.000
Interaction—epsilon W A 3.341
Interaction—epsilon W B 4.073
Interaction—epsilon W W 0.000

Molecule

Name P85
Architecture A 6 B 12 A 6

Molecule

Name Water
Architecture W 1

System

Name P85C24
Temperature 298.00
Grid 32 32 32 1.04
Composition P85 0.24 Water 0.76
Compressibility_parameter 10.000

Noise

Expansion_parameter 100.000
Method DENSITY
Distribution UNIFORM

Simulation_control

Time_steps 40000
Time_interval 50.0000
Shear_rate 0.0010
Shear_start 1
Shear_end 2000
Shear_flag 0
Processors 1 1 1
Topology_file P85C24.MesoDyn_top
Status_frame 1

Restart_frame 100
 Potential_frame 100
 Density_frame 100

PEO₂₆PPO₄₀PEO₂₆—Pluronic™ P85 with Hydrophobic Drug Molecules, Haloperidol

Bead—PEO

Name A
 Diffusion_coefficient 1.000E-07
 Bondlength 1.20
 Volume 0.30

Bead—PPO

Name B
 Diffusion_coefficient 1.000E-07
 Bondlength 1.20
 Volume 0.30

Bead—Water

Name W
 Diffusion_coefficient 1.000E-07
 Bondlength 1.20
 Volume 0.30

Bead—Haloperidol

Name H
 Diffusion_coefficient 1.000E-07
 Bondlength 1.20
 Volume 0.30

Interaction Energies

Interaction—epsilon A A 0.000
 Interaction—epsilon B A 7.300
 Interaction—epsilon B B 0.000
 Interaction—epsilon W A 3.341
 Interaction—epsilon W B 4.073
 Interaction—epsilon W W 0.000
 Interaction—epsilon H A 5.129
 Interaction—epsilon H B 2.787
 Interaction—epsilon H W 12.091
 Interaction—epsilon H H 0.000

Molecule

Name PL64
 Architecture A 6 B 12 A 6

Molecule

Name Water
 Architecture W 1

Molecule

Name Haldol
 Architecture H 1

System

Name P85_HAL_001
 Temperature 298.00
 Grid 32 32 32 1.04
 Composition PL64 0.759 Water 0.240 Haldol 0.001
 Compressibility_parameter 10.000

Noise

Expansion_parameter 100.000
 Method DENSITY
 Distribution UNIFORM

Simulation_control

Time_steps 20000
 Time_interval 50.0000
 Shear_rate 0.0010
 Shear_start 1
 Shear_end 10000
 Shear_flag 0
 Processors 1 1 1
 Topology_file P85_HAL_001.MesoDyn_top
 Status_frame 1
 Restart_frame 100
 Potential_frame 100
 Density_frame 500

References

- [1] Lam, Y.M. and Goldbeck-Wood, G. (2003) "Mesoscale simulation of block copolymers in aqueous solution: parameterisation, micelle growth kinetics and the effect of temperature and concentration morphology", *Polymer* **44**(12), 3593.
- [2] Fraaije, J.G.E.M., Van Vlimmeren, B.A.C., Maurits, N.M., Postma, M., Evers, O.A., Hoffmann, C., Altevogt, P. and Goldbeck-Wood, G. (1997) "The dynamic mean-field density functional method and its application to the mesoscopic dynamic of quenched block copolymer melts", *J. Chem. Phys.* **106**, 4260.
- [3] Saxton, W.O., Pitt, T.J. and Horner, M. (1979) "Digital image processing: the Semper system", *Ultramicroscopy* **4**, 343.
- [4] Lam, Y.M., Grigorieff, N. and Goldbeck-Wood, G. (1991) "Direct visualisation of micelles of Pluronic block copolymers in aqueous solution by cryo-TEM", *Phys. Chem. Chem. Phys.* **1**(14), 3331.
- [5] Mortensen, K. and Talmon, Y. (1995) "cryo-TEM and SANS microstructural study of pluronic polymer solutions", *Macromolecules* **28**, 8829.
- [6] Van Krevelen, D.W. (1976) "Cohesive properties and solubility", Chapter 7, *Properties of Polymers*, 2nd Ed. (Elsevier Health Science, Amsterdam), pp 129–159.
- [7] Subrahmanyam, C.V.S. and Suresh, S. (1999) "Solubility behaviour of haloperidol in individual solvents determination of partial solubility parameters", *Eur. J. Pharm. Biopharm.* **47**, 289.
- [8] Malmsten, M. and Lindman, B. (1992) "Self-assembly in aqueous block copolymer solutions", *Macromolecules* **25**, 5440.