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Specific Interactions Improve the Loading Capacity of Block Copolymer Micelles in Aqueous Media

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Block copolymer micelles find application in many fields as nanocarriers, especially in drug delivery. We report herein that specific interactions between hydrophobic guest molecules and core-forming segments can significantly improve the loading capacity of polymeric micelles. High loading capacities (>100% weight/weight of polymer (w/w_p)) were systematically observed for the encapsulation of probes containing weak carboxylic acid groups by micellar nanoparticles having poly[2-(dialkylamino)ethyl methacrylate] cores (i.e., particles whose cargo space exhibits antagonist weak base functions), as demonstrated by the incorporation of indomethacin (IND), ibuprofen (IBPF), and *trans*-3,5-bis(trifluoromethyl)cinnamic acid (F−CIN) into either poly(ethylene oxide)-*b*-poly[2-(diisopropylamino)-ethyl methacrylate] (PEO-*b*-PDPA) or poly(glycerol monomethacrylate)-*b*-PDPA (PG2MA-*b*-PDPA) micelles. The esterification of IND yielding to a nonionizable IND ethyl ester derivative (IND−Et) caused an abrupt decrease in the micellar loading capacity down to 10−15% w/w_p. Similar results were also obtained when IND was combined with nonionizable block copolymers such as PEO-*b*-polycaprolactone (PEO-*b*-PCL) and PEO-*b*-poly(glycidyl methacrylate) (PEO-*b*-PGMA). The existence of acid−base interactions between the solubilizate and the weak polybase block forming the micelle core was confirmed by ^{1}H NMR measurements. However, the incorporation of high numbers of hydrophobic guest molecules inside polymeric micelles can provoke not only an increase in the hydrodynamic size ($2R_H$) of the objects but also a substantial change in the morphology (transition from spheres to cylinders). The application of the Higuchi model showed that the probe release followed a diffusion-controlled mechanism, and diffusion coefficients (D) on the order of 10^{-18}−10^{-17} cm^2/s were determined for IND release from 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ + 100% w/w_p IND. Probe release from micelles with weak polybase-based cores can also be triggered by changes in the solution pH.

Introduction

The ability of amphiphilic block copolymers to self-assemble into ordered structures when dissolved in a selective solvent (i.e., a solvent thermodynamically good for one block and poor for the other) is well known.[1−6] Micellar aggregates are characterized by their unique core−shell architecture, where in an aqueous environment the hydrophobic blocks of the copolymer are segregated from the aqueous exterior to form the inner core, and the hydrophilic blocks form the corona or the outer shell.[7] These assemblies have been successfully tested in several applications as nanocarriers,[8] especially in drug delivery.[9−11]

Current knowledge of the synthesis and self-assembling behavior of macromolecules has allowed the preparation of tailor-made polymer assemblies (mainly spherical micelles and vesicles) with excellent stability, functionality, stimuli-responsiveness, biocompatibility, and loading capacity, among other features.[9,12] In this regard, considerable effort has focused on the enhancement of micellar loading.[9] Ideally, the solubility parameters of the solubilizate (probe or drug) and the core-forming polymer block should be of the same order to achieve high loading into micelles.[4,9] However, there is no universal core-forming segment because each probe or drug is unique, as also formerly stated by Eisenberg et al.[4,13,14] Therefore, it is important to develop systems in which the active molecule "matches" the micellar core in terms of compatibility (Flory−Huggins interaction parameter $\chi \approx 0$).

It is worth noting that high loadings in micelles (>70−100% w/w_p) have been rarely reported in the literature for the physical encapsulation (exclusively) of hydrophobic guest molecules. A remarkable example of optimal drug delivery performance is the 17β-estradiol/PEO-*b*-PCL system, for which loadings as high as 190% w/w_p were observed.[14] Conversely, if one considers another model drug for studying micelle formation such as indomethacin (IND), which is a potent nonsteroidal anti-inflammatory agent presenting important irritation of gastrointestinal mucosa and toxicity to the central nervous system as a consequence of high plasma levels, the referenced data demonstrate that micellar loading capacities remain well below 50% w/w_p (e.g., 6−14% w/w_p for PEO-*b*-poly(alkyl methacrylates),[15] 8−9% w/w_p for

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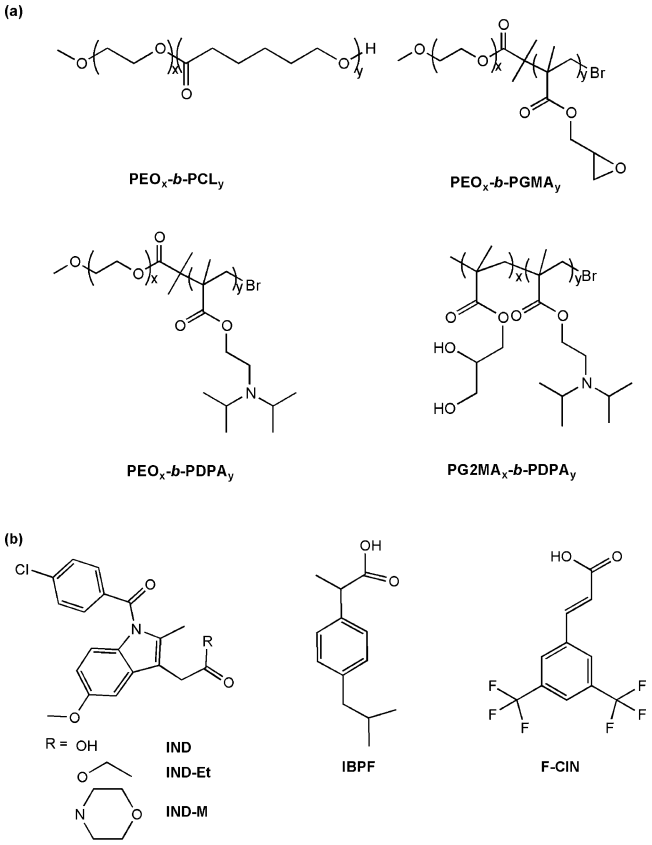
Scheme 1. Chemical Structure of the Diblock Copolymers (a) and Hydrophobic Probes (b)



PEO-*b*-poly(lactic acid),[16,17] 17−42% w/w$_p$ for PEO-*b*-polycaprolactone,[18,19] and 20−22% w/w$_p$ for PEO-*b*-poly(*β*-benzyl-L-aspartate)).[20]

In the present work, it is shown that specific interactions between hydrophobic guest molecules and polymers forming the micelle core can significantly improve the loading capacity of polymeric micelles. Proof-of-concept experiments were carried out combining structurally different hydrophobic probes (indomethacin (IND), indomethacin ethyl ester (IND−Et), indomethacin morpholinylamide (IND−M), ibuprofen (IBPF), and *trans*-3,5-bis(trifluoromethyl)cinnamic acid (F−CIN)) and block copolymers (poly(ethylene oxide)-*b*-polycaprolactone (PEO-*b*-PCL), poly(ethylene oxide)-*b*-poly(glycidyl methacrylate) (PEO-*b*-PGMA), poly(ethylene oxide)-*b*-poly[2-(diisopropylamino)ethyl methacrylate] (PEO-*b*-PDPA), and poly(glycerol monomethacrylate)-*b*-poly[2-(diisopropylamino)ethyl methacrylate] (PG2MA-*b*-PDPA)) (Scheme 1).

The outcomes suggest that probe/micelle systems exhibiting the ability to form R$_1$−COOH/NH$_2$−R$_2$ acid−base pairs within the micellar core display high loading capacities, which decrease considerably, for example, after esterification of carboxylic acid moieties. In fact, the concept of enhancing the micellar loading capacity through specific interactions has been put forth previously.[21,22] Lee et al.[21] demonstrated that the papaverine—a hydrophobic molecule having a pyridine ring (weak base) in its structure—loading content in micelles whose core was formed by poly(lactic acid) increased from 4 to 18% w/w$_p$ when a statistical hydrophobic copolymer containing carboxylic acid groups was used. The latter was synthesized by ring-opening copolymerization of lactide and 3-(*s*)-[(benzyloxycarbonyl)-methyl]-1,4-dioxane-2,5-dione (BMD) monomers, followed by deprotection of carboxylic acid groups in the BMD structure. The authors attributed the improvement in the loading capacity to hydrogen bonding between the polymer and the drug, whereas the existence of acid−base interactions, which can take place in that system on the basis of the respective pK_a values, was not considered. In an another approach, Nakanishi et al.[22] reported that satisfactory amounts of doxorubicin (DOX) were physically entrapped inside spherical micellar aggregates having poly(aspartic acid-*stat*-doxorubicin aspartate) (P(Asp-*stat*-DOXAsp)) cores. The loading capacity and stability of such micellar systems, which are currently in clinical trial development, were attributed to favorable P(Asp-*stat*-DOXAsp)-DOX interactions. Those authors noted, however, that the conjugated DOX fraction does not show biological activity with respect to cancer treatment.[22,23]

In the present work, high probe loading capacities were achieved using pH-responsive core-forming homopolymer blocks. The samples investigated hereafter can be synthesized via convenient atom-transfer radical polymerization (ATRP) protocols not requiring further chemical modification prior to micellization in order to obtain micellar systems with high loading capacities. The present approach is of broad interest inasmuch as it can be extended to various applications (drugs, cosmetics, pesticides, flavor masking, etc.).

Experimental Section

Materials. α-Methoxy-*ω*-hydroxy poly(ethylene oxide) (CH$_3$O-PEO$_{113}$-OH, Fluka, M_n = 5000 g/mol, M_w/M_n = 1.02), α-bromoisobutyryl bromide (Aldrich, 98%), *N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, Aldrich, 97%), 2,2′-bipyridyl (bpy, Aldrich, 99%), copper bromide (CuBr, Aldrich, 99.995%), *N*,*N*′-dicyclohexylcarbodiimide (DCC, Fluka, 98%), 4-dimethylaminopyridine (DMAP, Aldrich, 98%), indomethacin (IND, Fluka, 99%), indomethacin morpholinylamide (IND−M, Sigma-RBI), ibuprofen (IBPF, Aldrich, 98%), *trans*-3,5-bis(trifluoromethyl)cinnamic acid (F−CIN, Aldrich, 98%), and poly(ethylene oxide)-*b*-polycaprolactone block copolymers (PEO-*b*-PCL, Polymer Source Inc.) were used as received. Monomers 2-(diisopropylamino)ethyl methacrylate (DPA, Scientific Polymer Products, 99%) and glycidyl methacrylate (GMA, Aldrich, ≥ 97%) were distilled under reduced pressure before polymerization. Glycerol monomethacrylate (G2MA) monomer was kindly donated by Röhm Methacrylates and was used as received. THF (J.T. Baker) was purified by distillation over CaH_2 and then from a purple Na/benzophenone solution.

Synthesis of Indomethacin Ethyl Ester (IND−Et). IND−Et was prepared by Steglich esterification as described in the literature.[24] Briefly, IND (2.0 g, 5.6 mmol) was dissolved in 20 mL of ethanol under a nitrogen atmosphere. Subsequently, DMAP (0.07 g, 0.56 mmol) was added to the solution. After 10 min of stirring at 0 °C, DCC (1.15 g, 5.6 mmol) dissolved in 1.5 mL of ethanol was added dropwise. Subsequently, the solution was stirred for 30 min at 0 °C and then overnight at room temperature. The product was isolated first by removing the solvent under reduced pressure. Dichlo-

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romethane (30 mL) was added to the residue, and the suspension was filtered to remove solid byproducts (urea). The filtrate was extracted with saturated K_2CO_3 (3 × 10 mL) aqueous solution, and the organic phase was dried with anhydrous $MgSO_4$, filtered, and evaporated. The product (IND−Et) was purified by column chromatography (silica gel) using ethyl acetate and cyclohexane (1:1 v/v) as the eluent. The isolated product was obtained as a yellow solid (62% yield). ^{1}H NMR 400 MHz in $CDCl_3$ (δ): 7.66 and 7.46 (AB, 2H and 2H), 6.96 (d, 1H), 6.86 (d, 1H), 6.67 (dd, 1H), 4.15 (q, 2H), 3.83 (s, 3H), 3.65 (s, 2H), 2.38 (s, 3H), 1.27 (t, 3H).

Synthesis of Amphiphilic Diblock Copolymers. Amphiphilic diblock copolymers were synthesized by atom-transfer radical polymerization (ATRP).[25] The experimental conditions depended on the monomer being polymerized, and a detailed description for each case is given in the Supporting Information (SI, section 1). Briefly, PEO-*b*-PDPA and PEO-*b*-PGMA were prepared from bromo-terminated poly(ethylene oxide) macroinitiators (PEO-Br)[26] using, respectively, CuBr/HMTETA catalyst in THF at 60 °C and CuBr/PMDETA catalyst in diphenyl ether (DPE) at 30 °C.[27] The sequential monomer addition technique was employed to prepare PG2MA-*b*-PDPA diblocks using CuBr/bpy as the catalyst in MeOH at 20 °C. In all cases, [initiator]/[metal]/[ligand] = 1:1:2.

Polymer Characterization. *Nuclear Magnetic Resonance (NMR).* ^{1}H NMR spectra (400 MHz) were acquired using an Avance DPX 400 spectrometer. $CDCl_3$, MeOD, or THF-d_8 was used as the solvent, unless otherwise specified.

Gel Permeation Chromatography (GPC). Number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) values were determined by GPC either in THF (for PEO-*b*-PDPA, PEO-*b*-PDEA and PEO-*b*-PGMA) at a flow rate of 1.0 mL/min using a PLgel 5 μm mixed-C column on a Jasco apparatus equipped with a refractive index detector or in DMF (for PG2MA-*b*-PDPA) containing 1.0 g/L LiBr at a flow rate of 1.0 mL/min using a series of two PLgel 5 μm mixed-C columns, also on Jasco equipment. The calibration was performed using a series of nearly monodisperse polystyrene (PS) standards in both cases.

Sample Preparation. *Unloaded Micelles.* Dilute aqueous micellar solutions (C_p = 0.5 − 3.0 mg/mL) were prepared using the indirect dissolution or cosolvent method. Typically, 7.0 mg of diblock copolymer was added to a closed vial containing 0.3 mL of an organic solvent (either THF or ethanol for PEO-*b*-PDPA and PEO-*b*-PGMA, THF for PEO-*b*-PCL, and ethanol for PG2MA-*b*-PDPA) that is thermodynamically good for both segments constituting the polymers indicated. After stirring for at least 3 h, micellization was induced by the slow addition (0.33 mL/min) of 7.0 mL of Milli-Q water. Subsequently, the solution was stirred overnight or purged gently with N_2 for ca. 12 h to speed up the evaporation of organic solvents. ^{1}H NMR spectra of micellar solutions recorded before (control) and after such a procedure revealed the complete disappearance of chemical shifts associated with protons in the THF structure.

Loaded Micelles. An appropriate amount of probe (5−200% w/w_p) was dissolved along with the block copolymer in the organic medium, and the loaded micelles were prepared using essentially the same procedure as described above. After micellization (induced by the slow addition (0.33 mL/min) of Milli-Q water) and elimination/evaporation of the remaining organic solvent, the unloaded fraction of the hydrophobic probe that precipitated out of the solution was removed by filtration using $\varnothing$ = 0.45 μm pore size nylon filters.[28] Finally, the drug content inside the micelle was determined by UV−vis spectroscopy as described below.

Determination of the Loading Content in the Micelles. The probe content encapsulated inside the micellar carriers (core) was determined by UV−vis spectroscopy using analytical curves obtained at probe concentrations ranging from 5.0 to 100.0 μg/mL in THF or ethanol. In all cases, the UV−vis absorption intensity at λ_{max} depended linearly on the probe concentration. The probe loading content present in the original micellar solution was then determined after the dilution of 50 or 100 μL aliquots in 2.0 mL of THF or ethanol, thus provoking the demicellization of polymer chains (SI, section 2). The loading efficiency and the loading content were calculated using eqs 1 and 2, respectively.

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

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

In Vitro Release Kinetics. Release experiments were conducted using the dialysis method. In a typical experiment, 7.0 mL of a nanoparticles solution was sealed in a dialysis bag (Spectrum, MWCO = 25 000 g/mol), which was immersed in 2.0 L of a 0.025 mol/L phosphate buffer solution at pH 7.4. This buffer solution was periodically changed to ensure the release kinetics under nearly sink conditions. To follow the release kinetics, aliquots (50 μL) were withdrawn regularly from the micellar solution and diluted to 2.0 mL with THF or ethanol, thus causing the disassembly of block copolymer micelles. The released content was determined directly by UV−vis spectroscopy using the typical λ_{max}.

Dynamic Light Scattering (DLS). DLS measurements were performed using an ALV laser goniometer, which consists of a 22 mW HeNe linearly polarized laser operating at a wavelength of 632.8 nm and an ALV-5000/EPP multiple τ digital correlator with 125 ns initial sampling time. The copolymer solutions were maintained at a constant temperature of 25.0 ± 0.1 °C in all experiments. The accessible scattering angles range from 15 to 150°. The solutions were placed in 10-mm-diameter glass cells. The minimum sample volume required for DLS experiments was 1.0 mL. Data were collected using ALV Correlator Control software, and the counting time varied for each sample from 300 to 900 s. In the sequence, the relaxation time distributions, $A(t)$, were obtained using CONTIN analysis[29] of the autocorrelation function, $C(q, t)$. The relaxation frequency, Γ ($\Gamma = \tau^{-1}$), generally depends on the scattering angle, and in the case of a diffusive particle, this frequency is q^2-dependent.[30] Consequently, the apparent diffusion coefficient (D_{app}) at a given copolymer concentration (C_p) is calculated from

$$\frac{\Gamma}{q^2}\Big|_{q\to 0} = D_{app} \quad (3)$$

where q is the wavevector defined as

$$q = \frac{4\pi n}{\lambda}\sin\left(\frac{\theta}{2}\right) \quad (4)$$

λ is the wavelength of the incident laser beam, and θ is the scattering angle. The hydrodynamic radius (R_H) (or diameter $2R_H$) is calculated from the Stokes−Einstein relation

$$R_H = \frac{k_B T}{6\pi\eta\Gamma q^2} = \frac{k_B T}{6\pi\eta D_{app}} \quad (5)$$

where k_B is the Boltzmann constant, T is the temperature of the sample, and η is the viscosity of the medium.

Transmission Electron Microscopy (TEM). TEM images were recorded using a CM 120 Philips microscope operating at 120 kV, and equipped with a USC1000-SSCCD 2k × 2k Gatan camera. To

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Table 1. Molecular Characteristics of the Diblock Copolymers and Their Self-Assembled Structures

diblock copolymer[a]	M_n (g/mol)	M_w/M_n	$\phi_{hydrophobic}$[d]	$2R_H$[e] (nm)	pH-responsive	cosolvent for micelization
PEO_{45}-*b*-PCL_{24}	4700[b]	1.19	0.58	20	no	THF
PEO_{113}-*b*-$PGMA_{50}$	12 100[b]	1.10	0.59	42	no	THF
PEO_{113}-*b*-$PDPA_{12}$	7600[b]	1.18	0.34	20	yes	THF, EtOH
PEO_{113}-*b*-$PDPA_{50}$	15 700[b]	1.15	0.68	26	yes	THF, EtOH
$PG2MA_{40}$-*b*-$PDPA_{15}$	9600[c]	1.17	0.33	25	yes	EtOH

[a] Subscripts refer to the mean degree of polymerization (DP) of each block. [b] Determined by ^{1}H NMR measurements in $CDCl_3$ using the initiator methoxy moiety as a reference. [c] Calculated from the conversion determined by ^{1}H NMR in MeOD considering quantitative initiator efficiency. [d] Volume fraction of the hydrophobic block assuming that the polymer density is equal to 1.0 g/mL. [e] Micellar hydrodynamic size assessed by DLS.

prepare the TEM samples, 5 μL of an aqueous solution of block copolymer micelles was dropped onto a carbon-coated copper grid, which was rendered hydrophilic by UV/ozone treatment. Excess micelle solution was gently removed using absorbent paper. Samples were negatively stained by adding a 5 μL droplet of 2% sodium phosphotungstate solution at pH 7.4, and the excess solution was again removed prior to drying under ambient conditions.

UV−Vis Spectroscopy. UV−vis spectra were recorded using a Varian Cary 300 UV−vis spectrometer. For the measurements, 2.0 mL of solution was placed in a 10 mm^2 quartz cell. All spectra were recorded after baseline correction for solvent from air-equilibrated solutions in the 300−450 nm wavelength range at a scan rate of 600 nm/min (0.1 s integration per 1.0 nm).

Results and Discussion

Synthesis and Self-Assembly Behavior of Diblock Copolymers. Linear diblock copolymers formed by different segments and compositions were investigated as micellar nanocarriers (Scheme 1). PEO-*b*-PGMA and PEO-*b*-PDPA diblocks were synthesized by ATRP using PEO−Br macroinitiators prepared by the quantitative esterification of PEO−OH with α-bromoisobutyryl bromide. The ATRP of GMA in DPE at 30 °C in the presence of Cu(I)Br/PMDETA as the catalyst and the ATRP of DPA in THF at 60 °C using Cu(I)Br/HMTETA as the catalyst exhibited excellent first-order kinetics and a linear increase in the number-average molecular weight (M_n) with monomer conversion, indicative of controlled/living polymerization. For the PEO-*b*-PDPA system, samples with distinct volume fractions of hydrophilic and hydrophobic components were obtained by adjusting the [initiator]/[monomer] ratio. The controlled character of G2MA and DPA sequential polymerizations by ATRP in MeOH using Cu(I)Br/bpy as the catalyst was previously reported in the literature.[31] G2MA was polymerized first using a hydrophilic initiator (1-*O*-(2′-bromo-2′-methylpropionoyl)-2,3-*rac*-glycerol).[32] Once the monomer conversion was virtually complete, the second monomer (DPA) was introduced into the medium, consequently continuing the chain growth to produce PG2MA-*b*-PDPA diblocks.

The characteristics of the diblock copolymers are summarized in Table 1. The polydispersities remained below 1.19 even up to high conversions in all cases. Narrow molecular weight distributions are important in the field of macromolecular self-assembly in order to originate well-defined structures in solution. The thermodynamically stable morphology is defined mainly by the volume fraction (ϕ) of each constituting segment, along with the overall degree of polymerization (DP) and the Flory−Huggins interaction parameter (χ).[6] However, other factors such as the presence of additives might have considerable implications on the size and shape of polymer-based nano-objects.[33] Spherical core−shell micelles are favored for $0.30 < \phi_{hydrophobic} < 0.70$, whereas vesicles are expected for $\phi_{hydrophobic} > 0.70$.[6,34] In this work, the design of amphiphilic polymer chains exhibiting the ability to self-organize into spherical core−shell micellar morphologies was contemplated ($0.33 \geq \phi_{hydrophobic} \geq 0.68$, Table 1).

The aqueous micellar solutions investigated herein were prepared by the indirect dissolution method (also known as the cosolvent or dialysis method),[4,5] which was found to be appropriate for all samples listed in Table 1. The organic cosolvent used to prepare the micellar solutions depended on the solubility properties of the diblock copolymers. Except for PG2MA-*b*-PDPA, all the other samples could be molecularly dissolved in THF. PDPA-containing polymers dissolved easily in EtOH as well (Table 1). The use DMF (a good solvent for all samples) in these biomedical-related applications was avoided because of its well-known toxicity. Using either THF or EtOH, self-organization into spherical core−shell micelles was observed in all cases after the dropwise addition of water (a selective solvent for PEO and PG2MA) and the removal of the organic phase (Scheme 2). The hydrodynamic diameter ($2R_H$) of the resulting objects ranged from 20 to 42 nm, and the polydispersities were usually narrow ($\mu_2/\Gamma^2 = 0.08-0.20$ using DLS cumulants analysis), as illustrated in Figure 1 for PEO_{113}-*b*-$PGMA_{50}$ and PEO_{113}-*b*-$PDPA_{50}$ diblocks. The mean micelle diameters observed in TEM micrographs are clearly smaller than those determined by DLS measurements (PEO_{113}-*b*-$PGMA_{50}$ micelles (Figure 1a): $2R$(TEM) = 20 nm and $2R_H$(DLS) = 42 nm; PEO_{113}-*b*-$PDPA_{50}$ micelles (Figure 1b): $2R$(TEM) = 16 nm and $2R_H$(DLS) = 26 nm). This discrepancy is in part due to micelle dehydration caused by solvent evaporation under the high-vacuum conditions employed during TEM imaging experiments. However, discrepancies are also expected because DLS reports an intensity-average diameter, whereas TEM reports a number-average diameter. Thus, for a given size distribution and finite polydispersity, TEM images will usually undersize the micelle diameter as compared to DLS measurements.

Loading of Hydrophobic Guest Molecules Containing Carboxylic Acid Groups into Micelles Having Weak Polybase Cores. In the case of PDPA-containing polymers, stimulus-induced self-assembly can also be applied for micelle preparation because of the pH-responsiveness of the PDPA segment. As a weak polybase, PDPA can be molecularly dissolved in dilute acid solution because the DPA block is protonated and hence hydrophilic under these conditions. On adjusting the solution pH to around 6 to 7, the PDPA becomes deprotonated and hence hydrophobic, leading to the formation of micelles with dehydrated PDPA cores.[35] Although this solvent-free protocol seems very attractive from a micellization standpoint, it is not suitable for

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(33) Ouarti, N.; Viville, P.; Lazzaroni, R.; Minatti, E.; Schappacher, M.; Deffieux, A.; Borsali, R. *Langmuir* **2005**, *21*, 1180−1186.
(34) Discher, D. E.; Ahmed, F. *Annu. Rev. Biomed. Eng.* **2006**, *8*, 323−341.
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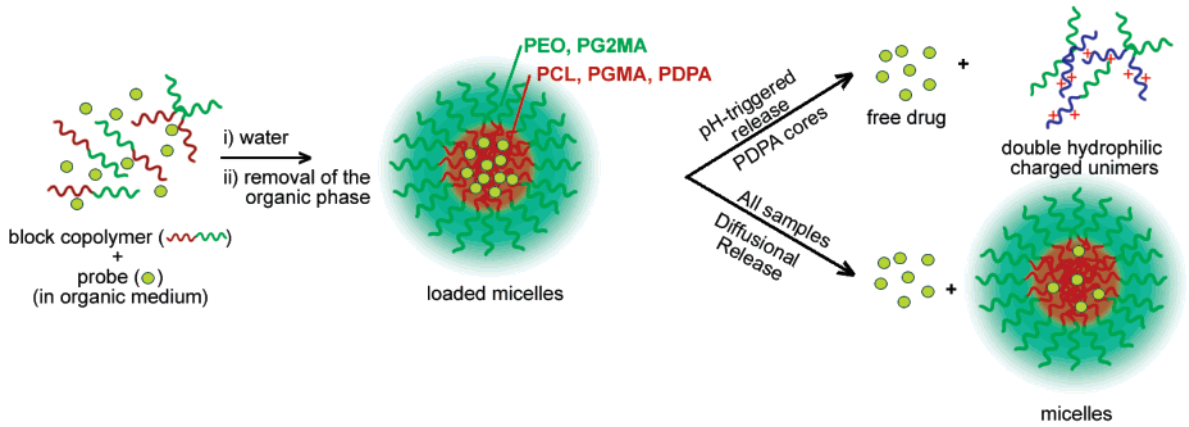
Scheme 2. Illustration Showing the Micellization via Indirect Dissolution Method and Possible Drug Release Mechanisms (pH-Triggered or Diffusional)



the encapsulation of hydrophobic guest molecules, which in most cases have to be dissolved in the organic medium along with the polymer prior to micellization in order to achieve acceptable loadings (>5% w/w_p). Instead, such a stimulus-responsive characteristic presents great potential for controlled release because pathological areas are generally characterized by local hyperthermia or acidosis. Consequently, micelles having stimulus-responsive core-forming blocks can release their payload within those regions via triggered-release processes (Scheme 2).[11]

In general terms, when a hydrophobic probe (or drug) and an amphiphilic block copolymer are dissolved together in an organic medium, then the addition of water (a selective solvent) to the latter induces the self-assembly of amphiphilic copolymer chains, possibly originating a range of morphologies (micelles, cylinders, vesicles, etc.) that can serve as containers for the encapsulation of a given fraction of drug. The unloaded part usually precipitates out of the solution and is removed by simple separation methods (centrifugation, filtration, etc.).

Remarkably high loading capacities have been observed for indomethacin (IND) incorporation into micelles having weak polybase cores (namely, PEO-*b*-PDPA and PG2MA-*b*-PDPA). The variation of the IND content encapsulated by PEO_{113}-*b*-$PDPA_{50}$ micelles as a function of the total mass of IND used for the preparation is shown in Figure 2 for different polymer concentrations (C_p = 0.25−2.0 mg/mL). The dotted line represents the quantitative loading, and the horizontal lines refer to 100% w/w_p probe contents inside the micelles (i.e., the concentrations (in mg/mL) of entrapped probe and polymer are equivalent). For the IND/PEO_{113}-*b*-$PDPA_{50}$ system, the drug loading was proportional to the number of micelles in solution. For 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ micellar solutions (Figure 2, squares), nearly quantitative encapsulation was observed as the amount of IND in the initial solution increased until target loadings of ∼150% w/w_p (1.5 mg/mL IND) were achieved. Above this point, the curve deviates from the dotted line, indicating partial encapsulation (up to ∼ 100% w/w_p; 1.0 mg/mL IND) regardless of the total amount of IND used. This behavior is also characteristic of diluted copolymer solutions (0.25 and 0.50 mg/mL). For 2.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ solutions (Figure 2, circles), however, IND was completely entrapped inside the micelles for target loadings lower than 50% w/w_p (1.0 mg/mL IND). However, the encapsulated content was further improved to 83% w/w_p (1.66 mg/mL IND) by increasing the IND amount in the initial solutions. Importantly, only in this later case the sedimentation of solids was observed typically after 1 week under steady (shelf) conditions. Otherwise, the solutions remained stable over periods of weeks, with no apparent material deposition.

The presence of a hydrophobic probe during the micellization of amphiphilic block copolymers affects the thermodynamics and kinetics of the process, leading to aggregates with distinct characteristics. For the IND/PEO_x-*b*-$PDPA_y$ case, micellar solutions underwent a noticeable visual change toward a bluish appearance as the IND content inside the nanocarriers increased. This is illustrated in Figure 2 (insets) showing digital photographs of 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ micelles encapsulating 100% w/w_p and 137% w/w_p IND in their cores. This behavior reflects either an important increase in the size of the particles or a transition in the morphology. The onset of this process depended on both the copolymer concentration and the PDPA block length, in agreement with earlier studies suggesting that the loading efficiency increases with the core-forming block.[9,13,14,28] For PEO_{113}-*b*-$PDPA_{12}$ and $PG2MA_{40}$-*b*-$PDPA_{15}$ (diblocks with short PDPA segments), such behavior was observed when the targeted loadings were increased from 25 to 50% w/w_p IND (data not shown).

For a better understanding of the effects of high hydrophobic loadings on the physical−chemical properties of the system, IND-loaded PEO_{113}-*b*-$PDPA_{50}$ micelles were investigated in more detail using light scattering (DLS) and microscopy (TEM) techniques. Figure 3 shows the variations of the micellar hydrodynamic micelle diameter ($2R_H$) as a function of the IND loading content for 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ solutions. The results revealed a slight decrease in the $2R_H$ values for low IND loadings (≤25% w/w_p) as compared to those for unloaded particles, suggesting that micelles become slightly more compact. Upon increasing the IND content above 25% w/w_p, the size gradually increased until a sharp rise from 33 to 52 nm when probe content finally varied from 99 to 137% w/w_p was achieved. Such a remarkable increase in the size of the nanoparticles was also confirmed by TEM. Micrographs recorded for 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ + 137% w/w_p IND solutions (Figure 4a) revealed the presence of much larger objects ($2R$(TEM) = 45 nm) as compared to the unloaded counterparts (Figure 1b; $2R$(TEM) = 16 nm). The effect of high IND loadings on the micelle properties is more pronounced at higher copolymer concentrations. Both DLS and TEM analyses carried out on 2.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ + 133% w/w_p IND solutions after adequate dilutions (Figure 4b) clearly demonstrated the coexistence of spherical and cylindrical morphologies. The formation of cylinders is favored under such circumstances, with a number of Y junctions sometimes terminated by nearly spherical caps, as observed in Figure 4b. Morphology transitions (from micelles to cylinders to vesicles) are the usual responses of block

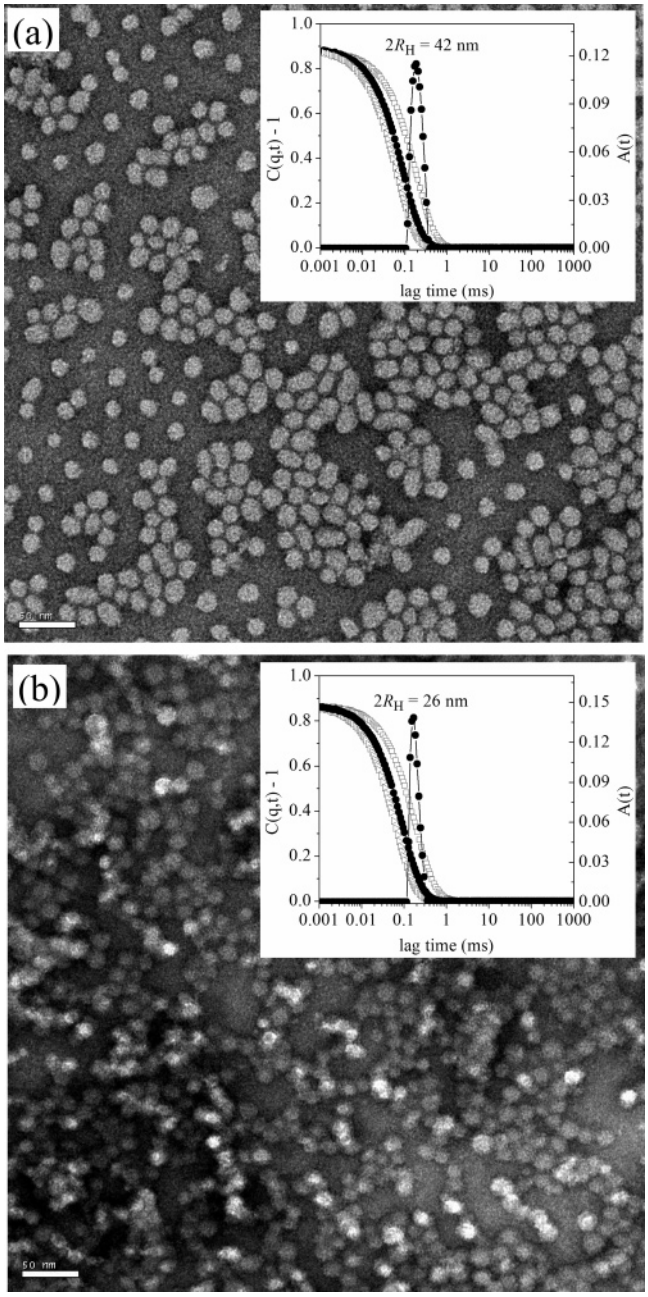


Figure 1. TEM images of unloaded PEO_{113}-*b*-$PGMA_{50}$ (a) and PEO_{113}-*b*-$PDPA_{50}$ (b) micelles. The insets show the corresponding autocorrelation functions $C(q, t)$ measured at scattering angles of 60° (□), 90° (●), and 120° (△) and distributions of relaxation times $A(t)$ at 90° as revealed by CONTIN analysis. The scale bar is 50 nm.

copolymers systems to increases in their solvophobic character, either in organic or aqueous media.[34,36]

Therefore, the results discussed above suggest that the morphology of block copolymer aggregates may change as a result of the presence of hydrophobic guest molecules, hence highlighting a different strategy to induce morphological changes in block copolymer systems. Nevertheless, this phenomenon represents a significant drawback in drug delivery applications because the size of nanodelivery systems should ideally remain below 200 nm in order to avoid the body defense mechanisms.[4]

Among the micellar systems with $2R < 200$ nm already described in the literature, the ability to encapsulate IND molecules was markedly lower than for the PEO-*b*-PDPA system (∼100%

(36) Jain, S. J.; Bates, F. S. *Science* **2003**, *300*, 460−464.

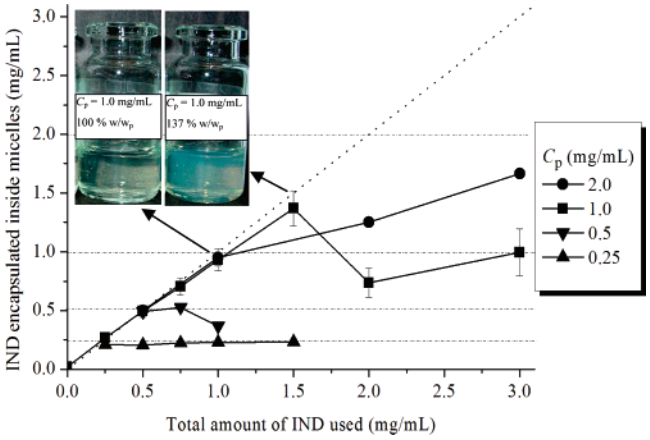


Figure 2. Amount of IND loaded into PEO_{113}-*b*-$PDPA_{50}$ micelles as a function of the amount used for different polymer concentrations, as indicated.

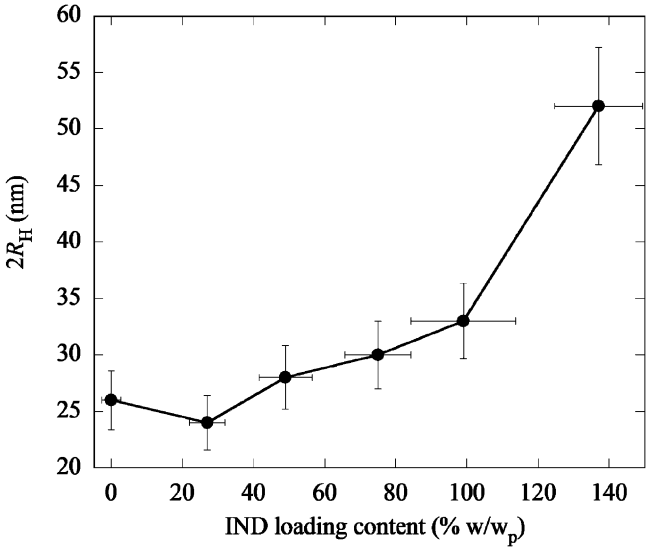


Figure 3. Variations of the hydrodynamic micelle diameter ($2R_H$) as a function of IND loading content for 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ solutions.

w/w$_p$ IND; stable, clear solutions). Namely, Leroux et al.[15] reported that 6−14% w/w$_p$ IND was encapsulated by micelles made from PEO-*b*-poly(*t*-butyl methacrylate) (PEO-*b*-P*t*BuMA), PEO-*b*-poly(ethyl acrylate) (PEO-*b*-PEA), and PEO-*b*-poly(*n*-butyl acrylate) (PEO-*b*-P*n*BuA) diblock copolymers. Using poly(lactic acid) (PLA) as the core-forming block, Choi and Kim[16] achieved IND loadings of 8 to 9% w/w$_p$ in PEO-*b*-PLA micelles, whereas the use of polycaprolactone (PCL) or poly(β-benzyl-L-aspartate) (PBLA) as the hydrophobic block revealed slightly higher IND loading capacities for PEO-*b*-PCL (17−42% w/w$_p$) and PEO-*b*-PBLA (ca. 20% w/w$_p$) micelles, as reported by Lee et al.[18,19] and Kataoka et al.,[20] respectively. In the mentioned systems, the IND molecules were physically entrapped inside the micelle core. Using a different approach, where poly(ethylene oxide)-*b*-poly(glycerol monomethacrylate)−IND conjugates (PEO-*b*-(PG2MA−IND)) were used as nanocarriers, the total IND loading (i.e., covalently bound (B−IND) + physically entrapped (F−IND)) was improved to 58% w/w$_p$, but a morphology transition from spherical micelles to large vesicles occurred above 50% w/w$_p$ IND.[37] Nanoparticles with enhanced IND loading

(37) Giacomelli, C.; Schmidt, V.; Borsali, R. *Macromolecules* **2007**, *40*, 2148−2157.

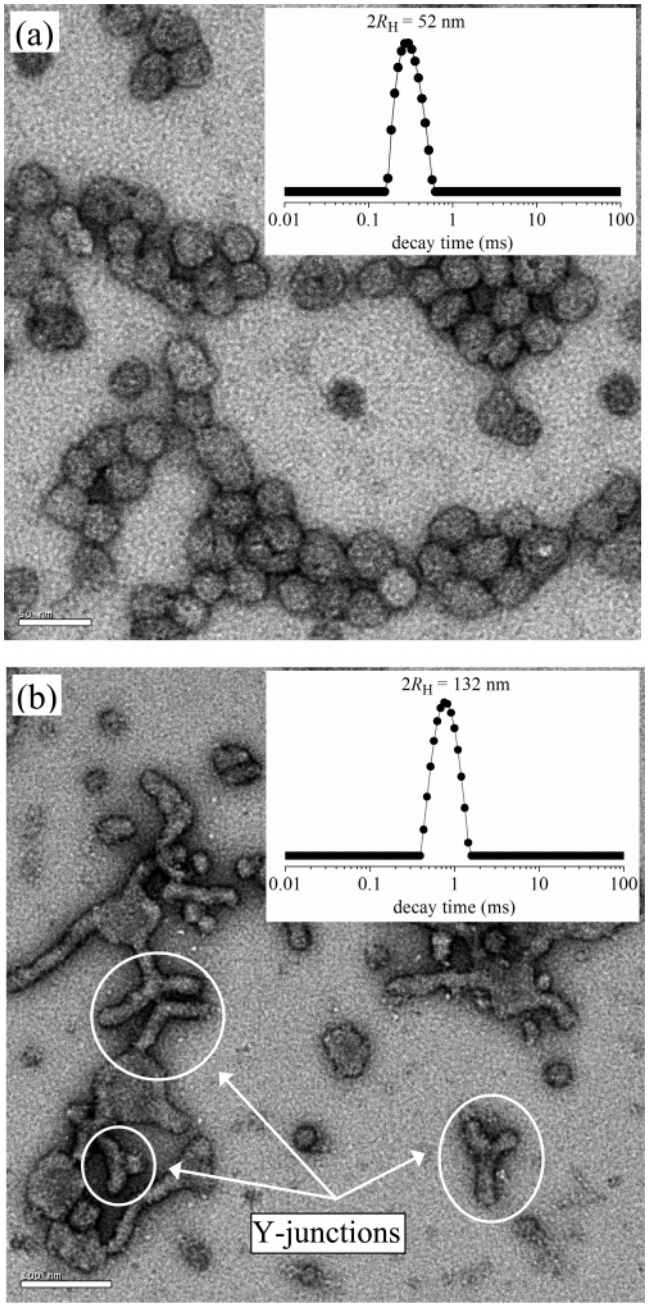


Figure 4. TEM images of highly IND-loaded PEO_{113}-*b*-$PDPA_{50}$ micelles: 1.0 mg/mL micelles + 137% w/w_p probe (a) (scale bar = 50 nm) and 2.0 mg/mL micelles + 133% w/w_p probe (b) (scale bar = 200 nm). The insets show the respective distributions of relaxation times $A(t)$ at 90° obtained using CONTIN analysis of the DLS results.

were also designed by Bertin et al.,[38] who synthesized block copolymers via ring-opening metathesis polymerization (ROMP) with sequential monomer addition of α-norbornenyl indomethacin and α-norbornenyl hexa(ethylene glycol) monomers. The resulting amphiphilic block copolymer−drug conjugates contained a high density of covalently bound IND (30−60% w/w_p). However, the self-assembly of these conjugates originated rather large particles ($2R$ = 130−1600 nm).

When other hydrophobic guest molecules such as ibuprofen (IBPF) and *trans*-3,5-bis(trifluoromethyl)cinnamic acid (F−CIN) (Scheme 1) were tested with PEO-*b*-PDPA and PG2MA-*b*-PDPA diblock copolymer micelles, the results revealed essentially the same behavior as described above for the IND/PEO-*b*-PDPA system (Table 2).

(38) Bertin, P. A.; Watson, K. J.; Nguyen, S. T. *Macromolecules* **2004**, *37*, 8364−8372.

Table 2. Loading Results for the Encapsulation of Hydrophobic Guest Molecules by Diblock Copolymer Micelles Having Different Core-Forming Segments

diblock copolymer[a]	probe	acid-base pair	added probe (% w/w_p)	encapsulated probe (% w/w_p)
PEO_{45}-*b*-PCL_{24}	IND	no	50	14
			100	13
	IND-Et	no	100	12
PEO_{113}-*b*-$PGMA_{50}$	IND	no	50	06
	IND	no	100	07
	IND-Et	no	100	06
PEO_{113}-*b*-$PDPA_{50}$	IND	yes	100	99
	IBPF	yes	100	100[b]
	F-CIN	yes	100	100[b]
	IND-Et	no	100	15
	IND-M	no	100	negligible

[a] C_p = 1.0 mg/mL. [b] Visual inspection; stable solutions with no apparent precipitation.

Therefore, micelles having weak polybase-based cores such as poly[2-(dialkylamino)ethyl methacrylate], present an optimized loading capacity for hydrophobic guest molecules containing antagonist carboxylic acid groups. To the best of our knowledge, micelle loading capacities as high as 100% w/w_p have been rarely reported in the literature for the physical encapsulation (not chemically linked) of hydrophobic molecules by well-defined block copolymer spherical micelles with $2R$ < 200 nm. An interesting result was reported by Soo et al.[14] for the 17β-estradiol/PEO-*b*-PCL system, for which the micelle loading was as high as 190% w/w_p.

Origin of High Micellar Loading Capacities. Ideally, the solubilizate should match the core-forming polymer in order to achieve high loading in micelles.[4,13] The reasons for the high loading capacities observed during the encapsulation of hydrophobic guest molecules containing carboxylic acid groups by polymeric micelles having weak polybase-based cores were investigated through the combination of structurally different probes and block copolymers (Scheme 1), in view of establishing a macromolecule structure-loading content relationship. The results are listed in Table 2 and clearly show that specific (acid−base) interactions between the guest molecules and the core-forming blocks are responsible for the enhanced micellar loading capacities. Among the probe/micelle systems studied in this work, those exhibiting the capability to form R_1−COOH/NH_2−R_2 pairs (indicated in Table 2) were able to stabilize high quantities of hydrophobic probes (∼100% w/w_p), as observed for IND/PEO-*b*-PDPA, IBPF/PEO-*b*-PDPA, and F−CIN/PEO-*b*-PDPA systems. The esterification of IND yielding to a nonionizable IND ethyl ester derivative (IND−Et) caused the maximum encapsulated contents to decrease abruptly to 15% w/w_p, being almost negligible for indomethacin morpholinylamide (IND−M). Similar results were also obtained when IND was combined with nonionizable block copolymers. For example, the substitution of PEO-*b*-PDPA by PEO-*b*-PCL or PEO-*b*-PGMA provoked a decrease in the loading capacity to ∼13% w/w_p or 6% w/w_p, respectively. Besides, no significant difference between IND and IND−Et loadings was evidenced in those cases.

The existence of specific acid−base interactions between the hydrophobic probes and the block copolymers was corroborated by ^{1}H NMR experiments. Figure 5 shows ^{1}H NMR spectra for 15 mg/mL IND in the absence (a) and in the presence of 16.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ (20% excess of DPA units) in THF-d_8, simulating the first step of the micelle preparation procedure

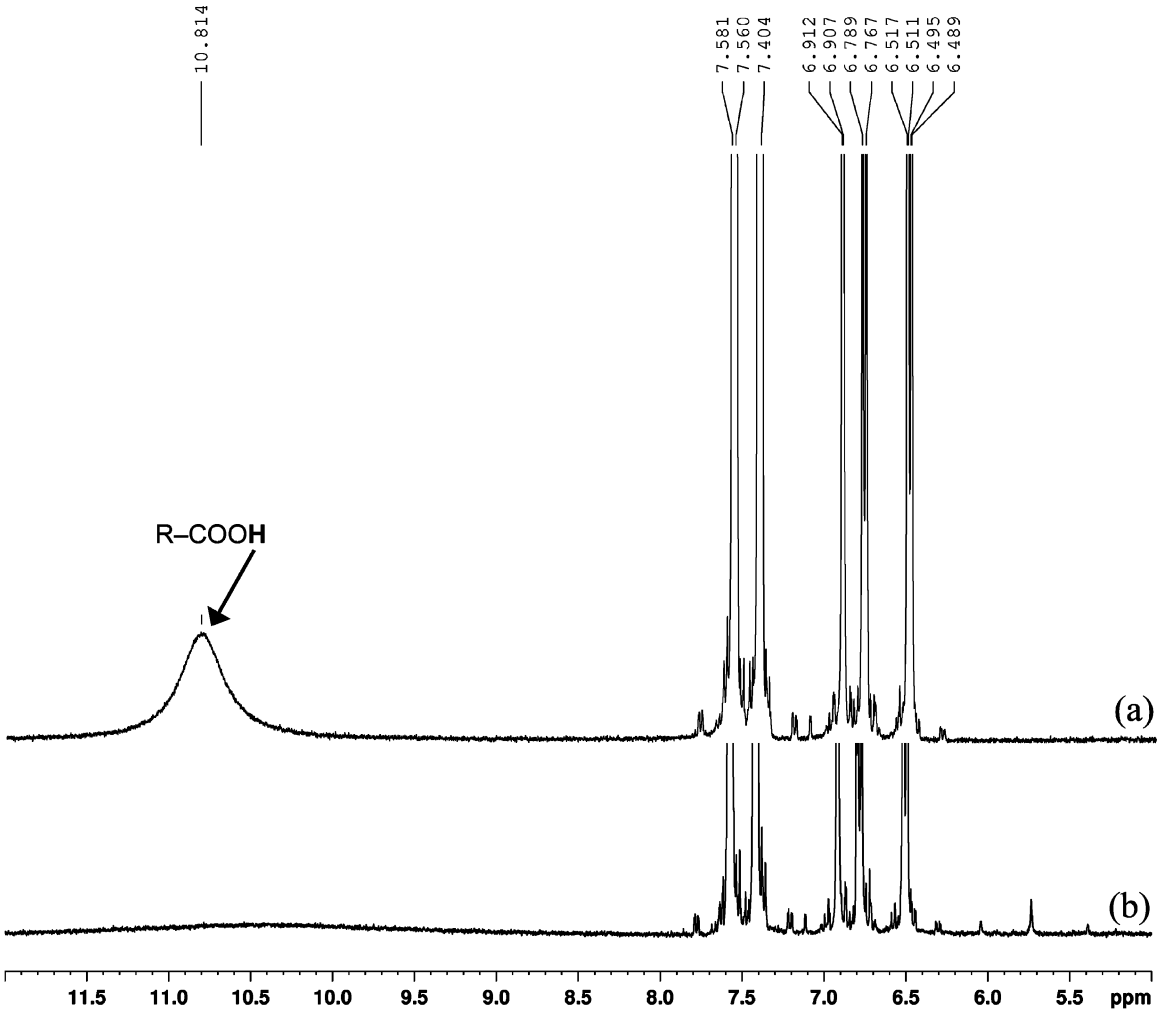
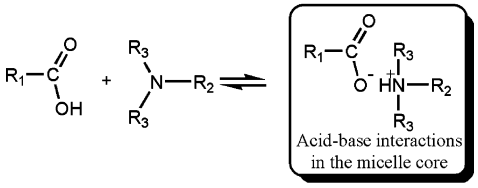


Figure 5. ^{1}H NMR spectra for 15 mg/mL IND in the absence (a) and presence (b) of 16.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ (20% excess of DPA units) in THF-d_8.

Scheme 3. Acid−Base Interactions Inside the Micelle Core



(i.e., after probe and copolymer dissolution in THF and before the addition of water). In this experiment, the chemical shift at $\delta \sim 11$ associated with the labile acid proton of IND (spectrum a) basically disappeared in the presence of PEO_{113}-*b*-$PDPA_{50}$ (spectrum b).

Therefore, the high loading contents of hydrophobic molecules containing carboxylic acid groups inside micelles whose core is formed by weak polybases are ascribed to acid−base interactions (Scheme 3). The system can be modeled as the neutralization of a weak acid (guest molecule) with an antagonist weak base (micelle core), yielding to an inner structure similar to that of polyion complex (PIC) micelles.[9] This can be anticipated from the pK_a values of the drug to be encapsulated (e.g., pK_a(IND) = 4.5[20,39] and pK_a(IBPF) = 4.5[40]) and 2-(dialkylamino)ethyl methacrylate units (e.g., pK_a(PDPA-core) = 5.7).[28]

In Vitro Release Studies. The release of IND from highly loaded PEO-*b*-PDPA and PG2MA-*b*-PDPA micelles can be triggered by lowering the solution pH below the pK_a of PDPA because micellar dissociation occurs rapidly under such conditions. The pH-triggered release of IND is shown in Figure 6 for a 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ + 100% w/w_p IND solution and corroborates by visual inspection the existence of high amounts of drugs inside the micellar cores. The solution pH in this experiment is originally around 7.4 (right) and was lowered to 3.0 (left) by the addition of 1.0 mol/L HCl. Upon the dissociation of PEO-*b*-PDPA micelles due to the protonation of the PDPA block at low pH, the hydrophobic IND molecules precipitated out of solution.

However, sustained drug release kinetics is normally sought in order to decrease both the administration frequency and high plasma levels. The results of in vitro release studies are shown in Figure 7, which depicts a typical Higuchi plot for solutions containing 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ + 100 w/w_p IND. The inset displays the percentage of IND released over time. The linearity observed in this plot is indicative of a diffusion-controlled release mechanism, as proposed by Higuchi's model.[41] The latter has been previously used to fit the release of hydrophobic probes from block copolymer micelles[13,14,28,37] and is defined by eq 6, where Q is the amount of drug released per unit area of micelles, C_0 is the initial drug concentration per volume of core-forming block (expressed in mol/cm^3), and t is the time

(39) Nokhodchi, A.; Javadzadeh, Y.; Siahi-Shadbad, M. R.; Barzegar-Jalali, M. *J. Pharm. Pharm. Sci.* **2005**, *8*, 18−25.

(40) Wan, H.; Holmen, A.; Nagard, M.; Lindberg, W. *J. Chromatogr., A* **2002**, *979*, 369−377.

(41) Higuchi, T. *J. Pharm. Sci.* **1961**, *50*, 874−875.

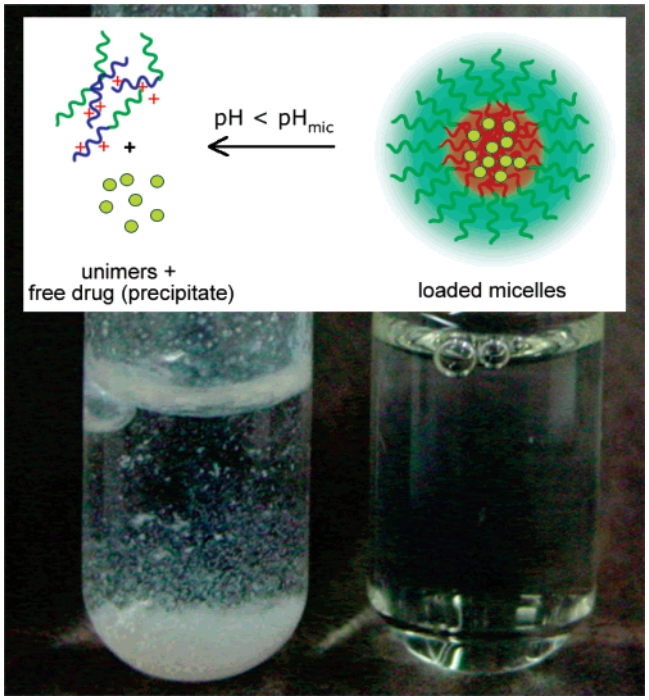


Figure 6. Digital photograph showing the pH-triggered release for a 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ + 100% w/w_p IND solution.

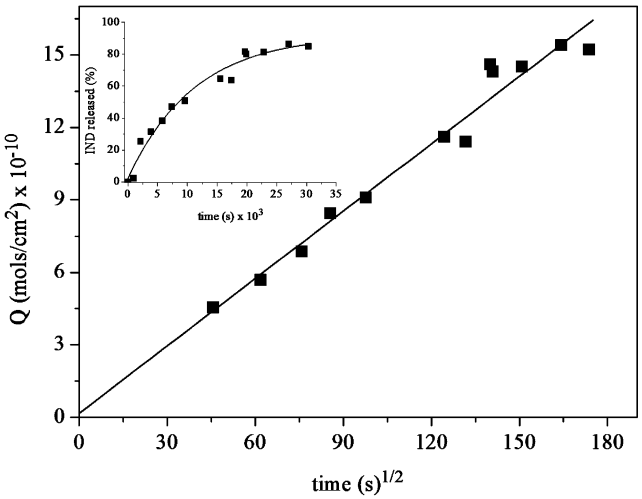


Figure 7. Probe release as a function of (time)$^{1/2}$ for 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ micelles prepared with an initial IND loading of 100% w/w_p at pH 7.4. The data correspond to an average of two independent measurements.

(expressed in seconds). A detailed description of its use was given recently.[14]

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{1/2} \quad (6)$$

The diffusion coefficient (D) extracted from Figure 7 was 6.4 $\times$ 10^{-18} cm^2/s, assuming that the micelle diameter is 33 nm (Figure 3) and that the density of PDPA is 1.0 g/mL. Comparable D values were also reported by Soo et al.[14] for highly 17β-estradiol-loaded PEO_{45}-*b*-PCL_{23} micelles ($D = 8.9 \times 10^{-18}$ cm^2/s). Furthermore, according to the results shown in the inset of Figure 7, the amount of drug released within an 8 h period from 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ + 100 w/w_p IND micellar solutions was approximately 85%, with almost complete release being achieved after ~24 h. This rather rapid release (in the absence of serum or protein) is probably associated with the dissociation behavior of IND. In fact, physically encapsulated IND has a carboxylic acid group whose pK_a is 4.5.[20,39] As a result of its aqueous solution behavior, neutral (IND) and negatively charged (IND^-) species coexist in solution, and the respective molar fraction depends on the solution pH.[20,37] The overall aqueous solubility constant (K_s) of IND is, for this reason, strictly determined by its degree of protonation. Although it can be considered to be a practically insoluble drug at pH 1.2 (K_s = 0.011 mmol/L), it is slightly soluble in pH 7.2 buffered solutions (K_s = 2.1 mmol/L).[39] The obvious difference in terms of K_s between IND and IND^- species has been claimed by Kataoka et al.[20] to explain the higher release rate (>15-fold) from micellar nanocarriers at neutral pH as compared to that in acidic media. In the present case, the release at pH < pK_a(probe) cannot be accessed because of the pH-induced demicellization of PDPA-containing aggregates (Figure 6).

The release profiles observed for highly loaded PDPA-containing micellar nanoparticles emphasize their excellent potential as delivery vehicles, as judged from their outstanding capacity to encapsulate, retain, transport, and deliver IND, IBPF, and F−CIN molecules.

Conclusions

Specific interactions between hydrophobic guest molecules and polymer segments forming the micelle core can significantly improve the loading capacity of self-assembled block copolymer particles in aqueous media.

Micelles whose hydrophobic core was formed by poly[2-(dialkylamino)ethyl methacrylate] (i.e., weak polybases) were successfully loaded with high quantities (up to 137% w/w_p) of lipophilic probes containing antagonist carboxylic acid groups in their structure. The existence of acid−base interactions between the solubilizate and the PDPA block forming the micelle core was confirmed by ^{1}H NMR measurements and through encapsulation experiments carried out on systems that combined structurally different hydrophobic probes (IND, IND−Et, IND−M, IBPF, F−CIN) and block copolymers (PEO-*b*-PCL, PEO-*b*-PGMA, PEO-*b*-PDPA, PG2MA-*b*-PDPA). However, high micelle loadings can provoke not only an increase in the hydrodynamic size ($2R_H$) of the objects but also changes in the morphology (from spheres to cylinders). The application of the Higuchi model showed that the probe release followed a diffusional mechanism, and diffusion coefficients on the order of 10^{-8}−10^{-7} cm^2/s were obtained for IND release from 1.0 mg/mL PEO_{113}-*b*-$PDPA_{50}$ + 100% w/w_p IND. Probe release from micelles with poly[2-(dialkylamino)ethyl methacrylate]-based cores can also be triggered by changes in the solution pH.

Finally, the results reported herein suggest that the loading capacity of block copolymer micelles can be tailored through the rational design of core-forming blocks and hydrophobic guest molecules.

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Supporting Information Available: Synthesis of diblock copolymers and UV−vis analysis. This material is available free of charge via Internet at http://pubs.acs.org.

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