Self-assembling polyether-\(b\)-polymethacrylate graft copolymers loaded with indomethacin

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ABSTRACT
The amphiphilic graft copolymers containing polyether-\(b\)-polymethacrylate side chains are proposed as carriers for a nonsteroidal anti-inflammatory drug, indomethacin (IMC). Two series of copolymers, poly ((methyl methacrylate)-co-(poly(ethylene glycol methacrylate))-\(graft\)-poly(methacrylic acid)-co-(\textit{tert}-butyl methacrylate)) (P(MMA-co-(PEGMA-\(graft\)-P(MAA-co-tBMA)))) and poly((methyl methacrylate)-co-(poly(propylene glycol methacrylate))-\(graft\)-poly(methacrylic acid)-co-(\textit{tert}-butyl methacrylate))) (P(MMA-co-(PPGMA-\(graft\)-P(MAA-co-tBMA)))) were applied in the micellization process. The effects of grafting degree (5–15%), length of the graft polymethacrylic segments (29–186 units), composition (PEG vs. PPG), and content of acidic fraction (52–89%) were verified in the design of two different types of micelles (depending on polyether nature) with proper stability and controlled release of the drug. The methacrylic segment with MAA units resulted in negatively charged layer as it was indicated by measurements of zeta potential (from \(-2\) to \(-44\) mV). The sizes of particles were in the range of 160–225 nm. The type of polyether segment influenced on the content of the drug encapsulation (PEG 39% vs. PPG 93% at 48% of hydrophobic fraction). In vitro experiments demonstrated significantly larger IMC releasing at pH 7.4 than in acidic conditions. The lower drug release rates were monitored for copolymers with long side chains containing large content of acidic units, what enhanced particle stabilization and drug-polymer interactions. The drug release profiles were well fitted to the Higuchi model, suggesting diffusion mechanism. The results confirmed that the graft copolymers might be applied as the carriers releasing a various doses of drug with the rate, which can be adjusted by wider sort of structural parameters than is possible in linear amphiphilic copolymers.

GRAPHICAL ABSTRACT

1. Introduction
Micelles based on amphiphilic polymers have paid widespread attention as a potential carriers for poorly soluble drugs. Compared to the other drug delivery systems (DDS), polymeric micelles exhibit unique properties, which make them great candidates useful in biomedical field [1]. Particle
size (below 200 nm), topology (block, star, graft, dendrimer, hyperbranched), and composition, including hydrophilic/hydrophobic balance, are important factors, as well as the extra stimuli sensitivity (pH or temperature) is responsible for their smartness behavior [2]. The self-assembly process is mainly managed by the critical micelle/aggregate concentration (CMC/CAC). Below the CAC, macromolecules form unimer structures, but when concentration increases above the critical point the micellization/aggregation process takes place [3]. The stability of polymeric micelles in diluted solution (similar to bloodstream) is expected to avoid their disassembly before reaching target and starting therapeutic process, which can be adjusted by the carrier structure. Nowadays the self-assembling amphiphilic block copolymers with hydrophobic-hydrophilic core-shell structures are the most investigated micellar DDS [4]. In the DDS the most popular shell-forming polymer is poly(ethylene glycol) (PEG), while poly(D,L-lactide-co-glycolide) (PLGA), poly(D,L-lactide) (PLA), and poly(ε-caprolactone) (PCL) are core-forming [5,6]. In some cases the micelles are stabilized by crosslinking shells, as it was reported for poly(acrylic acid) (PAA) in the block copolymer with PCL [7] or poly(4-vinylpyridine)-b-polystyrene [8,9], giving the so-called Knedel/doughnut shape structures. The polymer micelles with hydrophobic core and ionic amphiphilic corona containing charged and uncharged units were spontaneously self-assembled from polystyrene-b-poly(4-vinylpyridine-co-N-ethyl-4-vinylpyridiniumbromide) [10], and poly(styrene-co-acrylic acid)-b-PAA [11]. The vesicle superstructures formed from the amphiphilic biocompatible and biodegradable diblock copolymer of PEG-b-poly(L-lactic-co-glycolic acid) have been applied as carriers for anticancer drugs, nucleic acids, or dyes [12]. Using different binary solvent mixtures the amphiphilic block copolymers of polystyrene-b-poly[AA-co-(methyl acrylate)] were associated into various morphological aggregates, including cauliflower-like ones in the mixture of water/acetone [13]. The triblock copolymers of PEG-b-PCL-b-PAA were self-assembled yielding micelles with the hydrophobic PCL cores loaded with hydrophobic doxorubicin, and the functional PAA subcoronas (clung to the core), which were used to carry cisplatin through covalent interaction [14]. The other triblock copolymers, such as polystyrene-b-poly(N-isopropylacrylamide)-b-PEG [15], polystyrene-b-poly(2-vinylpyridine)-b-PEG [16], polystyrene-b-poly(sodium 2-acryloamido-2-methyl-1-propanesulfonate)-b-PEG [17], and polystyrene-b-PAA-b-PEG [18], resulted in the core-shell corona structures with thermo- or pH-responsive shells. In the case of triblock copolymers with polystyrene segment in the center (as the micelle core), the outer blocks formed mixed shell of PEG and poly(4-vinylpyridine), whereas the latter one was modified to a hybrid shell [19]. PEG-b-poly(tert-butyl acrylate-co-acrylic acid) can be self-assembled into the polymer vesicles with tunable sizes at various conditions yielding the PEG corona and shell based on the statistical segment [20].

The aim of our studies was to design the carriers based on the amphiphilic graft polymethacrylates containing polyether side chains (PEG or PPG) extended with polymethacrylate segments containing MAA units (Scheme 1). IMC was selected as the model drug due to its chemical characteristic, anti-inflammatory, and analgesic properties. Previously reported self-assembling structures by our group, which were obtained from 6-hydroxyhexanoic acid 2-(2-methacryloyloxy)ethyl ester-based graft copolymers [21,22] and poly(ethylene glycol) methacrylate hydroxy-terminated graft copolymers [23], have indicated significant influence of the structural parameters, such as nonlinear topology, grafting density, and graft lengths. In the present work we postulate formation of micelles with different nature of polyether (hydrophilic PEG vs. hydrophobic PPG), and content of hydrophilic fraction of MAA units in the polymethacrylic block of the side chain–generating layer with ionizable groups. Our investigations were mainly focused on physicochemical parameters of the polymeric self-assemblies, concerning their micellar stability and ability for encapsulation of drug in via hydrophobic interactions with the hydrophobic core. In this context, the main parameters, that is hydrophilic/hydrophobic ratio, CAC, zeta potential (ZP), and particle size, were evaluated, whereas the carrier properties were monitored by drug loading efficiency and kinetic release profiles.

2. Experimental

2.1 Materials

Indomethacin (IMC; 2-[1-[(4-Chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid) (Alfa Aesar, 98%); pyrene (Sigma Aldrich, 98%); acetate buffer solution (ABS), 1.0 M, pH 5.0 (Alfa Aesar); and phosphate buffer solution (PBS), 1.0 M, pH 7.4 (Sigma Aldrich) were used as received. All solvents were applied without purification. The amphiphilic graft copolymers with polyether-bl-polymethacrylic side chains (I-VI, Table 1) based on poly(ethylene glycol) methacrylate (PEGMA; Polysciences, Inc., MW = 520 g/mol) [24] or poly(propylene glycol) methacrylate (PPGMA; Aldrich, MW = 375 g/mol) [25] were delivered by our previous studies, which have been reported (synthesis details are given in the Supplemental Data).
Table 1. Characteristic parameters of the amphiphilic graft copolymers with polyether-bl-poly(MAA-co-tBMA) side chains.

| Graft copolymer | polyether | DP_{MAA}/DP_n | n_{MAA}/DP_{sc} | f_{hydrophob} |
|-----------------|-----------|---------------|-----------------|--------------|
| I               | PEG       | 10/215        | 100/122         | 0.36         |
| II              | PEG       | 10/215        | 126/186         | 0.48         |
| III             | PEG       | 8/178         | 23/39           | 0.64         |
| IV              | PPG       | 27/180        | 16/18           | 0.47         |
| V               | PPG       | 21/166        | 29/56           | 0.67         |
| VI              | PPG       | 10/156        | 16/29           | 0.73         |

DP_{MAA}, DP_n, DP_{sc}: the polymerization degrees of macromonomer (PEGMA or PPGMA), total repeating units in the backbone, and repeating units in the side chains; n_{MAA}: number of MAA units after acidolysis; f_{hydrophob}: content of hydrophobic fraction in the graft copolymer.

### 2.2. Preparation of drug-free and drug loaded particles

Micelles were prepared using dialysis method. The graft polymer (20 mg) without or with IMC (weight ratio of polymer: drug 1:1) were dissolved in THF and deionized water (2-fold excess relative to THF) was added dropwise under stirring and the systems were stirred within 24 h to let micelles form. Next, the solution was transferred into cellulose membrane and dialyzed for 48 h against deionized water.

The amount of IMC encapsulated in micelles was quantitatively determined by a UV-vis method. The calibration curve used for drug loading characterization was established by the optical absorption of IMC at different concentrations in THF. Drug loading content (DLC) was calculated from the following equation:

$$DLC[\%] = \frac{\text{weight of loaded drug in micelles}}{\text{weight of drug loaded micelles}} \times 100\%$$

### 2.3. In vitro drug release study

The IMC release from polymeric micelles was determined at 37°C in acetate buffer solution (pH = 5.0) and phosphate buffer solution (pH = 7.4). After lyophilization, the drug-loaded micelles were dissolved in buffer (2 mg/1 mL) and transferred to dialysis tubes, which were immersed into 20 mL of the corresponding buffer and the media were stirred at 37°C within 72 h. At appropriate time intervals, aliquots of 2.5 mL release solution were taken out for measurements. The concentration of released drug was estimated by UV-vis spectroscopy at \(\lambda = 320.5\ nm\). or 60°C using a Malvern Zetasizer Nano-S90 equipped with an 4 mW He-Ne ion laser operating at \(\lambda = 633\ nm\) at a fixed scattering angle of 90°. At least four correlation functions were analyzed per sample in deionized water (1 mg/mL) in order to obtain an average value. Similarly, the surface charges of the polymer nanoparticles were measured in deionized water. Diluted samples (0.04 g/L) were placed in disposable folded capillary cell and put in the thermostatted cell compartment of the instrument (Malvern Zetasizer Nano-Z) at 25 ± 0.1°C. The measurements were carried out on sample from three independent runs.

### 3. Results and discussion

#### 3.1. Synthesis and micellization of graft copolymers

Two series of the well-defined graft copolymers with narrow molecular weight distributions, that is P[MMA-co-(PEGMA-graft-P[MAA-co-tBMA]) (I–III) (M_w/M_n = 1.15–1.39) and P[MMA-co-(PPGMA-graft-P[MAA-co-tBMA]) (IV–VI) (M_w/M_n = 1.25–1.44) varying on the lengths of polymethacrylic backbone (DP_{sc} = 178–215 and 156–180), degrees of grafting (DG ∼5% and 7–15%) and contents of hydrophobic domains (f_{hydrophob} = 0.36–0.64 and 0.47–0.73; Table 1) were selected for the micellization studies. The copolymers were composed of methacrylate backbone with statistically distributed polyether side chains (grafting degree controlled by the initial concentration of glycol macromonomer), which were extended by P retal (1c, 1d, 1e). These amphiphilic graft copolymers in aqueous solutions were enabled to form core-shell micelles, but polyether-b-polymethacrylic side chains provided differential superstructures depending on nature of the polyether segment (hydrophilic PEG vs. hydrophobic PPG). The PPG-based copolymers resulted in standard micelles with hydrophobic backbone and PPG segments in the core, whereas graft polymethacrylic segments with ionizable acidic units were placed in the shell (Scheme 1c, IV–VI). In the case of PEG-based copolymers the most probable were superstructures with core covered by mix shell consisting of the water-soluble PEG segments, which were back-folded by polymethacrylic segments with ionizable carboxyl and hydrophobic tert-butyl groups yielding loops, as depicted in Scheme 1c (I–III). The formation of flower-like aggregates was also achievable by the complexation (interpolymer complexes) between MAA units containing electron deficient groups and PEG segments with regions of high electron density [26]. The looping as a kind of the collapse of side chains was induced by the contracting polymethacrylic chains with MAA, what enhanced association between MAA and PEG chains in via hydrogen bonds. The effect of noncovalent association between groups of different polymer chains, which are based on hydrogen bonding, van der Waals interactions, and polyelectrolyte association as the results of thermodynamic preferences, are well known in the literature. The self-complexation between PEO/PEG and PMAA-based blocks via hydrogen bonding was postulated as the driving forces in the micelle formation.
by water-soluble poly(sodium methacrylate)-b-poly(ethylene oxide) [27] and PMAA-b-PEG-b-PMAA (with better stability in pH 4.8) [28]. Double hydrophilic block copolymers were self-assembled in water into stable micellar nanoparticles with poly(2-hydroxyethyl methacrylate) core and PEO shell (triblock copolymers with inner PEG block resulted in flower-like micelles) [29].

The CAC, which is one of the most important parameters related to the thermodynamic stability of micelles, was determined by the fluorescence spectroscopy with pyrene as a probe using intensity ratio of $I_{338}/I_{333}$ in the logarithmic function at the different concentrations of polymer (Figure 1). It is well known that the micelles based on linear block copolymers are formed at lower critical concentration in comparison to the standard low molecular weight surfactants [4], for example, sodium dodecyl sulfate in water forms micelles at 8 mol/L [30].

As it is shown in Table 2, the CAC values of copolymers with polyether-b-P(MAA-co-tBMA) side chains were ranged in 0.15–0.25 mg/mL (PEG as polyether) and 0.16–0.40 mg/mL (PPG as polyether). In the case of P(MMA-co-PEGMA) without polymethacrylic segments in the side chains the micelles were formed at lower critical concentrations (0.06–0.08 mg/mL) as it was reported in literature [13]. The micelles based on P(MMA-co-(PEGMA-b-P(MAA-co-tBMA))) graft copolymers I–III showed standard decreasing dependency of CAC with the increase in hydrophilic content (0.25 mg/mL at 36 wt% vs. 0.149 mg/mL at 64 wt%). In this series of copolymers the hydrophilic/hydrophobic balance was the main factor responsible for CAC changes, which were adjusted by the content of PEG and length of the polymethacrylic side chains with MAA units. Comparing the copolymers I and II with the same lengths of backbones and grafting degrees, the latter one was more proficient for the self-assembly due to larger hydrophobic fraction as a result of significantly longer polymethacrylic side chains (122 vs. 186 units) with slightly larger number of MAA units (100 vs. 126 units). Comparable CAC values at differential hydrophobic/hydrophilic ratios were obtained for copolymers II and III, which were drastically varying by lengths of side chains (DP_{sc} = 186 corresponding to $n_{\text{MAA}} = 126$ and 39/23, respectively). The results for self-assembling PPG-b-P(MAA-co-tBMA) graft copolymers IV–VI with various grafting degrees (7–15%) and comparable lengths of backbones confirmed correlation between the content of hydrophilic units in the polymethacrylic side chains and CAC, which was decreased with reducing number of hydrophilic MAA units in the side chain ($n_{\text{MAA}} = 29–16$). The copolymers IV and VI reached comparable CAC values at the same amounts of hydrophilic MAA units, although they contained the extreme amounts of hydrophobic fraction (47% vs. 73%). A larger number of acidic units in copolymer V reduced self-assembling ability, which was demonstrated by higher critical concentration. Both series of block graft copolymers corresponded to different self-assembly properties as the consequence of the polyether nature, hydrophilic PEG versus hydrophobic PPG, which generated various interactions between polymeric chains in the aqueous medium.

The measurements of ZP confirmed the presence of ionic surface layer becoming from acidic units in the prepared polymeric particles. The results exhibited strong correlation of this parameter with structure of the graft copolymer (Table 2). Two trends were distinguished, i) particularly negatively charged particles (ZP ≈ −40 mV) at higher content of hydrophobic fraction, that is above 45% for copolymers with PEG-b-P(MAA-co-tBMA) grafts (II, III) or 70% for copolymer VI with PPG-b-P(MAA-co-tBMA) grafts, and ii) other particles with nearly neutral surface (ZP ≈ −8 mV). Rationally, more negative values should be correlated to higher content of acidic units, but such a relationship was not observed for the studied graft copolymers, comparing I versus III ($n_{\text{MAA,I}}>n_{\text{MAA,III}}$ when ZP$_{I}$>ZP$_{III}$) or V versus VI. In both series of PEG versus PPG, some significant differences in structural parameters were remarked. The graft copolymer III with comparable grafting degree to I and II (~5%) distinguished by extremely short polymethacrylate segment with hydrophilic MAA units, whereas copolymer VI was characterized by twice lower grafting degree than in IV and V. Correlation of the surface potential with graft copolymer structure let to conclude that the extra factors are responsible for this unusual ionic layer properties.

According DLS analysis the largest particles were obtained for graft copolymers II and IV containing the longest...
backbones in both series, but the length of side chains forming the shell should be also considered. In the first sample the longest side chains with the largest amounts of MAA generated the strong hydrophilic interaction with aqueous solution, what was additionally intensified by the presence of hydrophilic PEG in the shell. However, in sample IV the polymethacrylic segments in side chains were the shortest, but they were almost completely acidic with ability of spreading out. The sizes of other particles were detected in narrow range of 165–190 nm. This means that the predomination of MAA units (60–80%) was not preferential factor in the interactions with aqueous media, because the presence of statistically distributed hydrophobic tBMA units (not transformed into MAA) probably destabilized the hydrophilic interactions. The representative histograms of the particle sizes are shown in Figure 2. It is also worth to remind that the miscellaneous interactions of graft copolymers in water yielded two types of core-shell micelles considerably varying on the structure of outer layer, that is MAA units versus mix of PEG and MAA units, which also influenced on the particle sizes.

### 3.2. Preparation of drug-loaded micelles and their characterization

The drug loading into hydrophobic core of the micellar particles helped to improve solubility of the drug, which enhances in vivo bioavailability of hydrophobic drug, whereas the hydrophobic interactions copolymer-drug and between drug molecules determine efficiency of the DLC. Nonsteroidal anti-inflammatory IMC (Scheme 1b) characterized by very poor water solubility was used for encapsulation by the self-assembling copolymers with polyether-b-P(MAA-co-tBMA) side chains to evaluate their potential application as the drug carriers. The DLC values for P(MMA-co-(PPGMA-b-P(MAA-co-tBMA))) based micelles were twice higher (40–93%) than for micelles with PEG as the polyether segment (18–39%) at comparable content of the hydrophobic fraction, as it was distinguished for II and IV at ~48% of Fhydrophob or III and IV at ~65% (Table 2). Although in the series IV–VI DLC was increased linearly with reduction of hydrophobic content. In the case of drug encapsulation by graft copolymers the influence of grafting density is remarkably probable. The copolymers I–III with the lower grafting degree (4–5%) indicated lower ability of drug loading than the copolymers IV–VI with larger number of side chains (15–6%). Additionally, low grafting degree of VI was corresponded to the lowest DLC value in the PPG-based graft copolymers, which was relatively closed to DLC of I–III containing PEG side segments. The drug loading effect was amplified proportionally to hydrodynamic diameter of the blank micelles. After drug encapsulation particle sizes slightly increased by about 10–25 nm (I, III, VI). Twice larger particles were detected for sample IV, which can be explained by the highest amount of loaded drug. The exceptions for II and V with the longest side chains in the both series of graft copolymers representing almost two times lower particles might suggest the shrinking behavior by partial entrapment of drug molecules in the shell.

### 3.3. IMC release profiles

The drug release rate usually depends on a various factors including polymer composition, topology, particle size, and

| Graft copolymer | CAC (g/L) | $D_h$ (nm) | PDI | ZP (mV) | DLC (%) | $D_{h,IMC}$ (nm) | PDI | IMC |
|----------------|----------|------------|-----|---------|---------|-----------------|-----|-----|
| I              | 0.252    | 174        | 0.196 | –2      | 17.9    | 189             | 0.179 |
| II             | 0.155    | 280        | 0.138 | –44     | 39.4    | 126             | 0.261 |
| III            | 0.149    | 164        | 0.146 | –40     | 23.3    | 190             | 0.268 |
| IV             | 0.161    | 225        | 0.138 | –3      | 93.3    | 453             | 0.548 |
| V              | 0.297    | 190        | 0.272 | –8      | 55.6    | 114             | 0.178 |
| VI             | 0.156    | 178        | 0.240 | –41     | 40.3    | 190             | 0.263 |

**Figure 2.** Size distribution profiles (by intensity) of nanoparticles based on graft copolymers III (blank [a], IMC loaded [b]) and VI (blank [c], IMC loaded [d]) by DLS measurements in aqueous solutions at 25°C.
hydrophilic/hydrophobic balance or pH medium. The kinetic profiles of IMC released from polymeric particles were performed in PBS (pH 7.4) and ABS (pH 5.0) as the simulated physiological solutions in experiments *in vitro* at 37°C within 72 h. As it can be seen in Figure 3, in all cases double higher amounts of IMC were released at pH 7.4 (micelles based on PEG-b-P(MAA-co-tBMA) graft copolymers: 24–56% and PPG-b-P(MAA-co-tBMA) graft copolymers: 44–71%) than in the acidic conditions (13–29% and 18–37%, respectively).

The faster IMC release observed at pH 7.4 seems to be rational due to gradual ionization of –COOH groups in MAA units improving their hydrophilicity, whereas in acidic solutions with pH 5, the protonation of polycarboxylate anions of MAA causes their increased capability for the intra- or intermolecular hydrophobic interactions. Comparing the release profiles (Figures 4a and 4b), the drug is easier released from micelles formed by copolymers with a shorter side chains as it was observed for the PEG-b-P(MAA-co-tBMA)–based systems I versus III (DPsc = 122 vs. 39), in which after 24 h the following values of free drug were detected, 17% versus 43% at pH 7.4 and 9% versus 20% at pH 5.0. In the case of PPG-b-P(MAA-co-tBMA) graft–based micelles IV versus V (DPsc = 18 vs. 56) there was not significant influence of the structure on the amount of released IMC (∼35% at pH 7.4 and 14% at pH 5.0 within 24 h), but at the lowest grafting density (6%) the sample VI indicated difference in relation to above systems of the same type (50% at pH 7.4 and 18% at

**Figure 3.** IMC release profiles within 72 h from (a) PEGMA-based and (b) PPGMA-based nanoparticles in different media (pH 7.4 and 5.0) at room temperature.

**Figure 4.** Zero-order plot of percentage of drug released in time from micelles based on graft copolymers of (a) PEGMA I versus III and (b) PPGMA V versus VI loaded with IMC in pH 7.4 and 5.0 at 37°C.
pH 5.0 within 24 h). All kinetic profiles demonstrated rather fast release in the first 5 hours, and next it was slowed yielding the additional 6–8% of free IMC within almost three days, excluding sample III, where double portion of the free drug was detected. Higher rate of drug release in the initial stage can be explained by a gradient concentration of drug, which apart from core resided in the interface up to the shell (amphipathicity effect), which has been also reported in literature for other systems [31]. However, the self-assemblies based on the PPG block graft copolymers showed higher predisposition for release of larger amount of drug, especially in neutral conditions, which suggests their lower stability than the mix PEG/MAA shell superstructures. In acidic environment larger amount of the released drug (>25% for systems of II, III, VI) was correlated to more negatively charged shell (ZP ≈ −40 mV). Additionally, pH-sensitive MAA units provided two conformations, the ionized extended chains at pH > 7 (due to the electrostatic repulsion) and the nonionized hypercoiled chains at acidic pH. Thus the obtained micellar systems with various rates of drug release responding to the pH environment may be suitable for oral or suppository delivery of IMC and other hydrophobic anti-inflammatory drugs, which after releasing in the stomach at acidic conditions are able to start therapeutic process in relatively longer time than in the intestine where they enter the bloodstream. Previously studied micellar systems formed from the branched copolymers indicated broad ranges of the released IMC amounts, which are ~45% by star-shaped AB3 type poly(ε-caprolactone)-b-P(M)AA) [32], 12–88% by copolymers based on 6-hydroxyhexanoic acid 2-(2-methacryloyloxy)ethyl ester with PMAA grafts [33], and 30–65% by hydroxy-functionalized PEG-grafted copolymers [22] at pH 7.4.

The release mechanism of drug from polymeric micellar structure is mostly based on diffusion of drug through the entangled chains, and erosion of polymer matrix, which are correlated with specific characteristics of drug delivery system, including ionization effect. The IMC release data were fitted to the most common kinetic models of the drug release amount (Q) in a function of time (t), which is zero-order kinetic, first-order kinetic, and Higuchi models. The drug release

|             | Zero order | First order | Higuchi model |
|-------------|------------|-------------|---------------|
| pH = 5.0   | pH = 7.4   | pH = 5.0    | pH = 7.4      |
| I           | 0.7665     | 0.7679      | 0.867         | 0.922         | 0.8923        | 0.9672        |
| II          | —          | 0.739       | 0.7978        | —             | 0.9101        |
| III         | 0.4722     | 0.6201      | 0.9205        | 0.8892        | 0.8509        | 0.9393        |
| IV          | 0.8867     | 0.8319      | 0.9417        | 0.8821        | 0.9792        | 0.9343        |
| V           | 0.5272     | 0.8547      | 0.7863        | 0.9071        | 0.8251        | 0.9733        |
| VI          | 0.7696     | 0.8363      | 0.9283        | 0.9504        | 0.8553        | 0.9805        |

Figure 5. The kinetics of IMC release at pH 7.4 and 5.0 at 37°C from micelles based on graft copolymers of PEGMA as a linear plot of log (% remaining drug) versus time (a) in accordance with the first-order equation and (b) a linear plot of % of released drug versus square root of time in accordance with the Higuchi square root model.
independent on concentration is described by the zero-order kinetic (Eq. 1), whereas the systems demonstrating concentration dependent drug release correspond to the first-order kinetic expressed by semilogarithmic plot (Eq. 2) \[34\]. The Higuchi describes drug release based on Fickian diffusion as the cumulative percentage of drug release vs. square root of time (Eq. 3) \[35\].

\[
Q = kt
\]

\[
\log(1 - Q) = -kt/2.303 \text{ or } \ln(1 - Q) = -kt
\]

\[
Q = kt^{1/2}
\]

where Q is the amount of drug released at time t and k is release rate constant.

The model fitting release data were evaluated by the correlation coefficients (R^2), which are collected in Table 3. The kinetic profiles in Figure 4 show that the drug release was not followed by the zero-order model, but in the case of systems IV (both pH) and VI (at pH 7.4) the regression values were relatively high (0.83–0.88). When the data were plotted according to the first-order equation the correlation was significantly higher (R^2 = 0.78–0.95; Figures 5a and 6a) indicating the concentration influence on drug release. The best fit was found with the Higuchi model (R^2 = 0.82–0.98; Figures 5b and 6b), where especially fair linearity was detected for samples I, V, VI (at pH = 7.4), and IV (at pH = 5.0) with correlation coefficient above 0.965. The latter correlation suggests that drug release in the studied systems was controlled by diffusion through the polymeric matrix.

### 4. Conclusions

In these studies, the graft copolymers containing polyether-b-P(MAA-co-tBMA) side chains were designed to achieve the self-assembles for the delivery of IMC. The combination of various segments, that is, the hydrophobic polymethacrylate backbone, hydrophobic PPG or hydrophilic PEG, and ionizable MAA units (in the alkaline environment), caused the formation of two types of micelles, that is the standard core-shell and the core-mix shell structures. The particles with adjustable stability revealed the broad range of IMC loading content (17–93%) dependent on structure parameters of the graft copolymers, such as hydrophobic/hydrophilic balance, lengths of backbone and side chains, grafting density, and acidic content. The increased hydrophobicity supported higher encapsulation of hydrophobic IMC into the micelles. The IMC release profiles indicated twice lower efficiency of the release process in acidic conditions than at neutral pH (13–37% vs. 24–71%) with significant contribution of diffusion process. The self-organized PEG-b-P(MAA-co-tBMA) and PPG-b-P(MAA-co-tBMA) graft copolymers provided a promising...
matrices for drug delivery due to their relatively low CAC values with ability for drug encapsulation, and graft topology enabling to regulate rate of drug release.

**Acknowledgments**

The authors also thank Professor Andrzei Dworak and MSc. Katarzyna Laba for possibility to use the fluorescence spectrophotometer in the Centre of Polymer and Carbon Materials (Polish Acad. Sci.).

**Funding**

This work was financially supported by the National Science Center (Grant No. N N204 122940). Paulina Maksym-Bębenek is a scholar under the project “DoktoRIS” cofinanced by European Union under the European Social Fund.

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