A Kinetic Model To Explain the Zero-Order Release of Drugs from Ionic Polymeric Drug Conjugates: Application to AMPS−Triflusal-Derived Polymeric Drugs

Alberto Gallardo,* Gema Rodríguez, María Rosa Aguilar, Mar Fernández, and Julio San Román

Instituto de Ciencia y Tecnología de Polímeros, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

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ABSTRACT: The microstructural characterization of metacryloyloxyethyl [2-(acetyloxy)-4-(trifluoromethyl)]benzoate (THEMA) and 2-acrylamido-2-metylpropanesulfonic acid (AMPS) copolymers (THA) is described. Both monomers presented a high differential reactivity in copolymerization (the reactivity ratios have been determined, $r_{THEMA} = 4.54$ and $r_{AMPS} = 0.32$), and the monomer incorporation to the copolymer chains is quite heterogeneous: the more reactive THEMA is incorporated preferentially in the first steps of the reaction and AMPS in last stages. Two rich THEMA copolymers (0.60 and 0.80 THEMA feed molar fraction), as well as the homopolymer, have been used for in vitro Triflusal (Disgren) release experiments, giving rise to zero-order profiles. A kinetic model called the "progressive accessibility model" has been developed in order to explain the zero-order kinetics of Triflusal release from AMPS−THEMA copolymers in different media, at pH = 2.0, 7.4, and 10.0. The experimental results fit adequately the model, which considers the accessibility of the hydrolyzable THEMA units controlled by the microstructural distribution of these units in copolymer sequences.

Introduction

The ability to inhibit thrombus formation on surfaces is an important aim for polymers used to make small-diameter vascular grafts and any other devices coming in contact with blood. In this sense, the synthesis of polymers with good haemocompatibility has permitted a reduction of the administration of heparin associated with the use of blood contact devices and, therefore, the reduction of the side effects derived from it, mainly thrombocytopenia and haemorrhage. Different strategies have been developed to improve the long-term efficacy of these vascular substitutes, being one of the most important the modification of the blood contact surfaces with active polymeric coatings. It has been recently described by our group that the application of specific polymeric drugs derived from acetylsalicylic acid on the inner surface of small diameter vascular grafts improves the prevention of adhesion and aggregation of platelets on the surface.[1−4] Polymeric drugs are simply insoluble prodrugs that can be converted to their active, soluble form through hydrolysis. Copolymers are a well-known approach to altering the physical properties of polymers and to obtaining a specific physicochemical and biological behavior. Their properties are controlled by the monomers incorporated in the macromolecules and by the chains microstructure. Our group has recently described the synthesis of statistical copolymers based on a methacrylic derivative of an antiaggregant drug, Triflusal (methacryloyloxyethyl[2-(acetiloxy)-4-(trifluoromethyl)] benzoate), and 2-acrylamido-2-methylpropanesulfonic acid (AMPS), a biocompatible monomer which incorporates biologically active sulfonic groups in its side chains. In this kind of system the drug is polymerized to form an insoluble matrix, and it permits the drug loading of up to 100% and ensure its long-term stability, activity, and bioavailability.

* Corresponding author: fax 34-915644853; e-mail gallardo@ictp.csic.es.

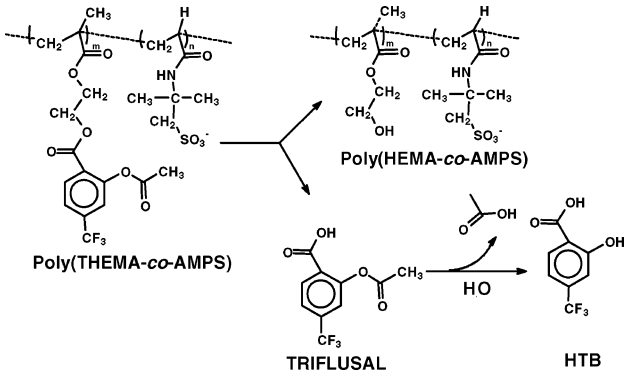

Figure 1. Scheme of the THEMA−AMPS copolymer structure and of the mechanism of hydrolysis and solubilization of the copolymer chains.

THEMA-rich copolymers were formulated in the range of water insolubility and therefore in the range of compositions useful for vascular grafty coating application. These insoluble polymeric formulations bearing Triflusal covalently attached as well as sulfonic groups prevented adhesion and activation of platelets.[4] These effects are due to the antiaggregant properties of Triflusal and, in addition, to the presence of the sulfonic groups which can mimic the antiacoagulant action of heparin.[5,6]

Figure 1 shows the release mechanism. Once Triflusal is released from the system, the HEMA−AMPS backbone becomes hydrosoluble, and on the other hand, free Triflusal is rapidly deacetylated and converted into the metabolite [2-hydroxy-4-trifluoromethyl]benzoic acid (HTB), which is the residue we analyze in the release experiments.

This article is devoted to the analysis of the poly(THEMA-*co*-AMPS) microstructure and to the study of the in vitro controlled Triflusal release at different pH. The in vitro drug release presented long-term zero-order kinetics, very interesting for this application owing to

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the fact that polymeric devices maintain the antiaggregant drug levels into the therapeutic levels for several months. This release behavior has been accurately described by a quite simple mathematical model named the "progressive accessibility model", which takes into account in its equations the microstructure of the polymeric devices.

Materials and Methods

Reagents. 2-Hydroxyethyl methacrylate (HEMA, Fluka) was exhaustively purified as described in the literature.[7] 2-Acrylamido-2-methylpropanesulfonic acid (AMPS, Avocado), [2-(acetyloxy)-4-(trifluoromethyl)]benzoic acid (Triflusal, Uriach), dioxane (Panreac), diethyl ether (SDS), and DMSO-d_6 (Merck) were used without further purification. Azobis(isobutyronitrile) (AIBN, Merck) was recrystallized twice from ethanol. Metacryloyloxyethyl[2-(acetyloxy)-4-(trifluoromethyl)]-benzoate (THEMA) was synthesized in our laboratory as described elsewhere.[1]

Polymers. Polymerization was carried out by a free radical mechanism as has been described previously:[4] both monomers were mixed in suitable proportions in order to obtain a final concentration of 0.3 M using water−dioxane (10:90) as solvent. After deoxygenation with gaseous N_2 for 30 min, the reaction was induced thermally at 50 °C by using AIBN (1.5×10^{-2} M) as free-radical initiator. After 24 h (total conversion) the solvent was removed by evaporation, and the isolated solid was redissolved in chloroform and precipitated in diethyl ether. The copolymers were filtered and dried under reduced vacuum until constant weight. The synthesized products were labeled as THA80 and THA60, depending on the feed molar composition of THEMA (0.80 and 0.60, respectively).

^{1}H NMR Monitoring of the Copolymerization Reaction. Reactivity ratios of the THEMA−AMPS system were determined following the method described elsewhere by Aguilar et al.[8] The copolymerization reactions were carried out inside the NMR equipment using DMSO-d_6 as solvent at 50 °C and AIBN as initiator. The global monomer concentration was 0.5 M, and the THEMA feed molar fractions were 0.8 and 0.6.

The experiments were carried out in a Varian 300 MHz spectrometer. In order to obtain quantitative data, a pulse sequence of 6 μs equivalent to a 90° tip angle and a 900 s (15 min) delay time was applied. One acquisition was used for each datum, nt = 1, to ensure instantaneous composition/conversion measurements. Sample temperature was maintained at 50 °C using the heater controller of the NMR equipment. A solution of (*N*,*N*-dimethylamino)pyridine (DMAP, 10 mg) in DMSO-d_6 charged in a thin wall capillary tube introduced in the NMR tube was used as reference.

Preparation of Polymer Films. Clear, transparent films (0.4−0.5 mm thick) were prepared by the casting method. A 30 wt % dissolution of the appropriate poly(THEMA-*co*-AMPS) in THF for THA80 or THF:ethanol (80:20) for THA60 was spread on a cylindrical mold of Teflon (15 mm diameter and 5 mm deep), dried at room temperature for 24 h, and finally dried overnight under reduced pressure until constant weight.

Swelling Behavior. The swelling behavior of THA80 and THA60 in buffered solutions at pH 2.0, 7.4, and 10 (1.5 M ionic strength) at 37 °C was monitored gravimetrically. The water uptake, $W = (\text{weight}_{wet} - \text{weight}_{dry})/\text{weight}_{dry}$, was calculated by measuring the weight gain of the sample at different times after wiping the surface carefully with filter paper. The experiments were done by triplicate.

Release Studies. Drug release in buffered aqueous media (pH = 2, 7.4, and 10) from THA80 and THA60 was followed using a HPLC apparatus, by monitoring Triflusal's main metabolite, [2-hydroxy-4-trifluoromethyl]benzoic acid (HTB), because of the poor stability of Triflusal in aqueous media.

The HPLC equipment had the following components: a Perkin-Elmer LC-250 pump, a UV detector Perkin-Elmer LC-95, and a Waters μBoundapak C-18 column of 3.0 × 300 mm. The experimental conditions for this assay were methanol/aqueous solution of PIC A (Waters) 0.1 M, (60:40) as mobile phase, 1 mL min^{-1} flow rate, and UV detection at 305 nm.

The polymers (25 mg, thickness 0.4-0.5 mm) were placed in stainless steel mesh envelops (allowing the films to be homogeneously in contact with the media) and immersed in buffered solutions (10 mL) at 37 °C. Samples were under continuous shaking. Portions of 0.5 mL of the supernatant were periodically collected for analysis and replaced with the same volume of fresh medium .

The release results of polyTHEMA have been previously described by our group.[1]

Results and Discussion

The synthesis, characterization, swelling, and some preliminary platelet adhesion experiments of THEMA-rich poly(THEMA-*co*-AMPS) have been recently reported.[4] This paper is devoted to the microstructural characterization and the analysis of the in vitro release profiles (including the development of a simple kinetic model) and their relationship with the microstructure of copolymer chains prepared with two different THEMA compositions, taken as model for the THEMA−AMPS copolymeric system.

Copolymerization reactions provide an excellent way for the preparation of macromolecules with specific chemical structures and for the control of the material properties. The reactivity and the particular characteristics of the reaction (mainly the control of the monomer addition during the growing of the macromolecular chains) can lead to dissimilar microstructures, which correspond to different sequence distributions. These differences can lead to particular molecular domains which, in the end, are biologically relevant.

The successive incorporation of the monomers to the growing chains and the chain microstructure in binary copolymeric systems following the terminal model (as the described in this work) are governed by the relative reactivity of monomers and the corresponding growing end radicals which in terms of kinetic parameter are expressed by the reactivity ratios r_{AMPS} and r_{THEMA}. These parameters have been calculated by the method described elsewhere by Aguilar et al.[8] The methodology uses the continuous change of the intensity of resonance signals assigned unambiguously to the monomers THEMA and AMPS with the reaction time. The evaluation of the monomer's concentration leads to the determination of the instantaneous feed molar fractions and the reactivity ratios, by using the integrated form of the differential copolymerization equation which describes the terminal model. We can apply the equation for a given reaction, taken different points as initial x_0, y_0 data, and a nonlinear least-squares fitting leads to the optimum $r_1 - r_2$ data depicted in Figure 2. The data exhibit a particular linear dependence for each experiment, the crossing point being the most reliable $r_1 - r_2$ data. The calculated reactivity ratios were $r_{THEMA} = 4.54$ and $r_{AMPS} = 0.32$. The composition diagram for this copolymeric system is shown in Figure 3. This graph gives us, according to the reactivity ratios, the instantaneous copolymer molar fraction for a given feed molar fraction. For instance, the polymerization of an equimolar mixture of monomers (F-feed = 0.5) at low conversion leads to the formation of macromolecular chains with a THEMA molar content of 0.8. Basically, THEMA is much more reactive than AMPS, and therefore, there is a tendency toward the formation of long THEMA sequences at the beginning of the reaction and long AMPS sequences at the end of the reaction.

Figure 4 shows the three-dimensional surface of the variation of the instantaneous THEMA molar fraction in the copolymeric chains as a function of the conversion

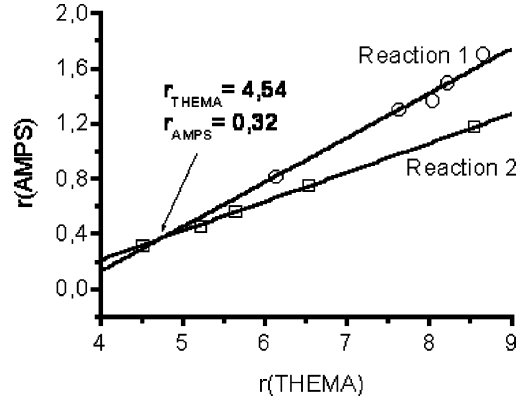


Figure 2. Plot of r_{AMPS} vs r_{THEMA} obtained as described in the text.

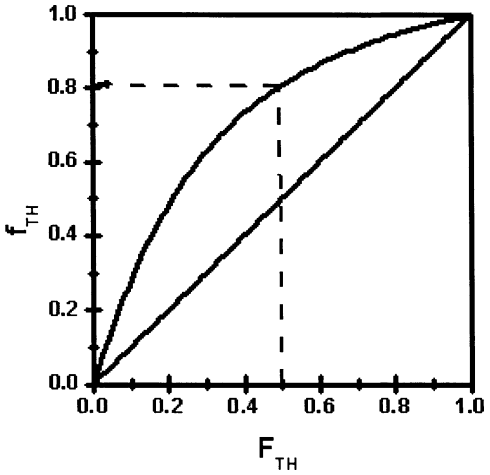


Figure 3. Composition diagram for the THEMA−AMPS copolymer system.

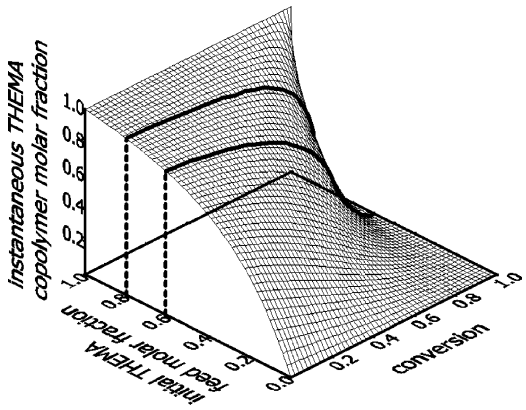


Figure 4. Three-dimensional surface of the instantaneous THEMA copolymer molar fraction vs the conversion and the initial THEMA feed molar fraction.

and the feed THEMA molar fraction, calculated as it has been described previously.[9] The more reactive unit, THEMA, is initially quickly consumed, and as a consequence, at low conversions THEMA-rich chains are formed. At high conversions, when most of THEMA has been consumed, there is a sharp transition toward the formation of AMPS-rich copolymer chains. Few chains with intermediate compositions are formed. The courses of the reactions used in this work have been marked with a solid line and have been selected as statistically representative examples of (initially) nonsoluble compositions.

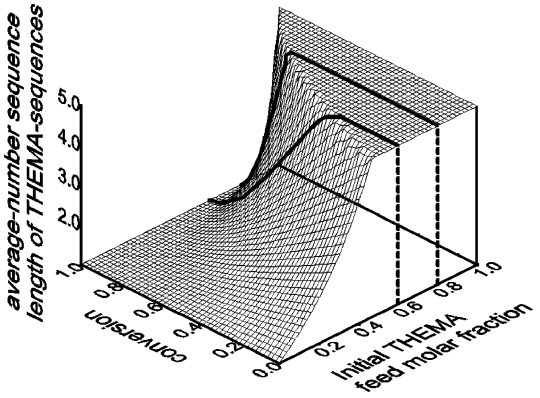


Figure 5. Three-dimensional surface of the instantaneous average-number sequence length of THEMA sequences vs the conversion and the initial THEMA feed molar fraction.

Figure 5 gives us additional information on the sequences distribution. In this graph, the average instantaneous sequence length of the copolymers calculated according to well-known expressions[10] are quoted versus conversion and initial feed compositions. Upper plateau is an artifact that represents the region with THEMA sequences longer than 5 units. The course of the reactions used in this work has been marked with a solid line. The copolymer chains formed in the first stages of the reaction are very rich in the more reactive monomer, THEMA, and average sequences up to 5 THEMA units are formed. These initial macromolecules incorporate few AMPS units that are isolated by long THEMA blocks. This initial block length strongly depends on the initial feed compositions, increasing with the initial THEMA feed molar fraction. As conversion increases, the THEMA sequence average length decreases. At high conversions (70−80%) residual AMPS begins to form long sequences.

In vitro release studies at basic, physiological, and acid pH have been carried out for the THA60 and THA80 copolymers. These copolymers exhibit a very interesting zero-order release, which at pH 7.4 is maintained for 6 months. This behavior seems to be very promising for a long-term activity of the coating, although this should be considered as an approximate correlation of the in vitro data with their in vivo performance. The zero-order behavior has been previously related[11] to the increasing hydrophilia for several acrylic polymeric drugs, simultaneous to the active component release because the macromolecular residue after the hydrolysis is a molecule with higher polarity than its parent. This also occurs in our case, since THEMA (hydrophobic) gives progressive rise to 2-hydroxyethyl methacrylate (hydrophilic) (see Figure 1).

The hydrolysis rate increases noticeably at alkaline pH (as it should be expected) because of the ester nature of the labile bond that undergoes the hydrolysis, which is catalyzed basically. Release at pH 2 is very low.

In addition, the copolymer composition exerts an important influence on the release behavior. The higher is the AMPS content and the hydrophilia of the matrix, the higher is the swelling (see Table 1) and the higher is the release rate because of the increased water accessibility to the labile ester bonds. The Triflusal release from the homopolymer is comparatively almost negligible.

Kinetic Model. THA copolymers have exhibited different release rates depending on the composition and the pH, and in all cases they followed a zero- or pseudo-

Table 1. Equilibrium Swelling Data and THEMA Copolymer and THEMA Centered Triad Molar Fractions (Cumulative)[a]

					equilibrium swelling %	
	f_{THEMA}	F_{ATA}	F_{ATT}	F_{TTT}	pH 2	pH 7.4, 10
THA60	0.630	0.076	0.308	0.617	125	153
THA80	0.810	0.027	0.185	0.789	30	49
polyTHEMA	1	0	0	1	2	3

[a] The copolymer molar fraction has been determined by ^{1}H NMR analysis as described elsewhere.[4] The triad data have been referenced to the THEMA copolymer molar fraction ($F_{ATA} + F_{ATT} + F_{TTT} = 1$) to enter in eq 6.

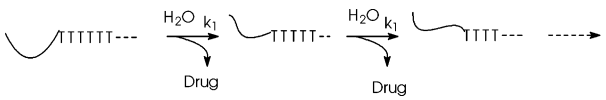
zero-order release for several months. It seems that the increasing hydrophilicity associated with the THEMA hydrolysis (and the conversion of hydrophobic THEMA in hydrophilic HEMA) is responsible for this "particular" kinetics, very interesting for a Triflusal-controlled release formulation. In fact, the residual skeleton HEMA−AMPS is water-soluble, and the solubilization takes place in some stages of the process.

Different models have been described in the literature in order to explain zero-order kinetics for polymer−drug conjugates. As an example, Rajewski et al. studied the hydrocortisone release mechanism from hyaluronic acid esters and developed a semiempirical mathematical model based on exponentially increasing polymer hydration, first-order ester hydrolysis, and rapid hydrocortisone diffusion.[12]

The THA copolymerization reaction gives rise to a very heterogeneous chain population because of the dissimilarities on the reactivity of the comonomers in free-radical reactions ($r_{THEMA} = 4.54$ and $r_{AMPS} = 0.32$). A detailed microstructural analysis, as described above, shows that a high concentration of THEMA and AMPS sequences is formed in the course of the reaction. Aggregation of these sequences can lead to the formation of microdomains.

As hydrophobic domains can be formed (THEMA-rich sequences), we can approach the problem from an accessibility point of view and not only as an obvious result of the increase in swelling. In a hypothetical hydrophobic domain, the outer ester groups are much more accessible to the hydrolytical agent (water), and the progressive drug release can be visualized as an unzipping process: the release and the formation of the hydrophilic residues open the hydrophobic domains and make accessible new ester functions. We have developed a very simple model based on this, called the "progressive accessibility model".

The model considers an unzipping mechanism for a THEMA sequence such as this:



(1)

The release of the outer THEMA unit makes accessible the neighbor one, keeping also the hydrolysis rate constant. From the reactivity ratios we can theoretically calculate the different sequence molar fractions by means of a method described elsewhere.[9] In Table 1, cumulative molar fractions of THEMA centered triads have been quoted.

Triflusal release has been divided into three contributions (Figure 6): (1) THEMA hydrolysis from the ATA triads, which have been considered accessible from the beginning (one order kinetic step); (2) THEMA hydrolysis from the ATT triads which evolve by the unzipping mechanism, keeping the ester concentration constant if it is broadly assumed that all the THEMA sequences have the same length (this approximation is necessary to simplify the model and to consider that step 3 takes place after step 2), until the consumption of the TTT triads (zero-order kinetics step); (3) THEMA hydrolysis from the residual ATT triads after the step 2 (exhaustion step, one order kinetics). According to this model, the second step is responsible for the zero-order release, and therefore, the higher is the TTT triads population (or the longer are the THEMA sequences), the higher is the zero-order character of the release. The rate in the zero-order regime is given by the number of active initial ATT triads. In the homopolymer there are not ATT or ATA triads, and the release rate predicted by the model is also zero, in good agreement with the experimental results. Steps 1 and 3 (exhaustion of the ATA and residual ATT triads) are one order with respect to the ester groups. This model also assumes a fast drug diffusion and that the water concentration associated with the AMPS domains is constant, and the differences in swelling are related to the hydrophobic/hydrophilic domains ratio. For that reason, the model includes the water concentration in the kinetic constant k_1.

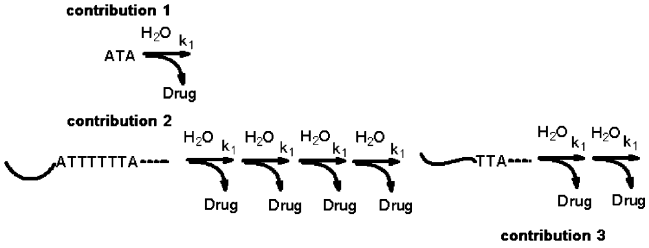


Figure 6. Scheme of the three contributions to the release taken into account by the model.

The kinetic expression for the drug release is given by

$$\frac{dn_{drug}}{dt} = k_1 n_{ATA} + \{\text{until consumption of TTT}\} \; k_1(n_{ATT})_0 + \{\text{from exhaustion of TTT}\} k_1 n_{ATT} \quad (2)$$

where n_i is the corresponding mole number of the i species (i = drug or any triad). The integration gives rise to

$$n_{drug} = (n_{ATA})_0 - (n_{ATA})_0 e^{-k_1 t} + \left(\text{if } t < \frac{(n_{TTT})_0}{k_1(n_{ATT})_0}\right), \; k_1(n_{ATT})_0 t + \left(\text{if } t > \frac{(n_{TTT})_0}{k_1(n_{ATT})_0}\right), (n_{ATT})_0 - (n_{ATT})_0 e^{[(n_{TTT})_0/(n_{ATT})_0]-k_1 t} + (n_{TTT})_0 \quad (3)$$

where $(n_{TTT})_0/k_1(n_{ATT})_0$ is the time at TTT exhaustion because

$$(n_{drug})_{contribution\ 2} = k_1(n_{ATT})_0 t$$

and at TTT exhaustion

$$(n_{drug})_{contribution\ 2,exhaustion} = (n_{TTT})_0 = k_1(n_{ATT})_0 t_{TTTexhaustion} \Rightarrow t_{TTTexhaustion} = \frac{(n_{TTT})_0}{k_1(n_{ATT})_0} \quad (4)$$

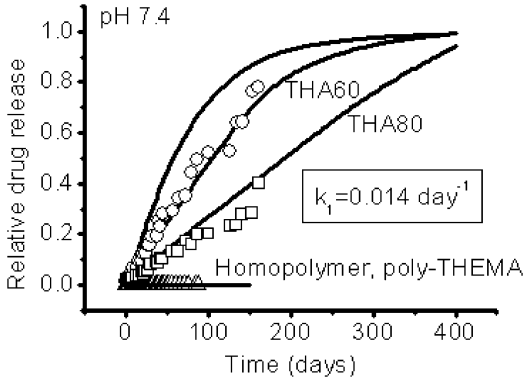


Figure 7. Release profiles of Triflusal from polyTHEMA and THEMA−AMPS copolymers at pH 7.4.

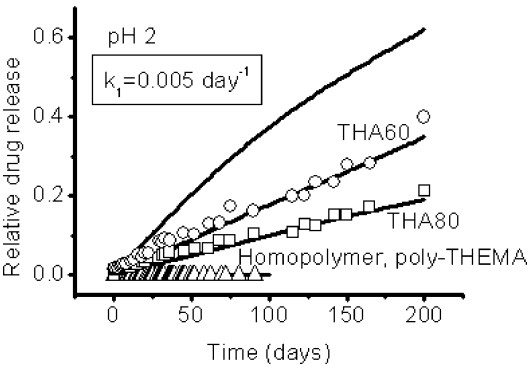


Figure 8. Release profiles of Triflusal from polyTHEMA and THEMA−AMPS copolymers at pH 2.

Then the release rate (Rrate) can be expressed as

$$\text{Rrate} = \frac{n_{\text{drug}}}{(n_{\text{THEMA}})_0} = \frac{n_{\text{drug}}}{(n_{\text{ATA}})_0 + (n_{\text{ATT}})_0 + (n_{\text{TTT}})_0} \quad (5)$$

and we can switch to THEMA centered triad molar fraction

$$\text{Rrate} = (f_{\text{ATA}})_0 - (f_{\text{ATA}})_0 e^{-k_1 t} + \left(\text{if } t < \frac{(f_{\text{TTT}})_0}{k_1 (f_{\text{ATT}})_0}\right), \; k_1 (f_{\text{ATT}})_0 t + \left(\text{if } t > \frac{(f_{\text{TTT}})_0}{k_1 (f_{\text{ATT}})_0}\right), \; (f_{\text{ATT}})_0 - (f_{\text{ATT}})_0 e^{[(f_{\text{TTT}})_0/(f_{\text{ATT}})_0] - k_1 t} + (f_{\text{TTT}})_0 \quad (6)$$

where

$$f_{\text{ATA}} = \frac{(n_{\text{ATA}})_0}{(n_{\text{ATA}})_0 + (n_{\text{ATT}})_0 + (n_{\text{TTT}})_0}, \text{ etc.}$$

The experimental Triflusal release points at the three pHs were fitted to this eq 6 by a least-squares method (see Figures 7−9). A single k_1 (0.005, 0.014, and 0.13 day^{-1} for pH 2, 7.4, and 10, respectively) was able to describe the release behavior of both copolymers and the homopolymer, polyTHEMA, in the in vitro experiments for several months. This fact supports (to some extent) the validity of the model.

The upper curve of Figures 7−9 corresponds to a reference case (for the same k_1 than the other curves) where no restricted accessibility exists (TTT triad population is zero), and the release follows first-order kinetics. It can be seen that the release rate decreases and the linearity of the release (that is, the zero- or pseudo-zero-order character) increases with the THEMA concentration and with the TTT triad content as was predicted by the model. The inner TTT triads of the THEMA blocks behave as a drug reservoir and contributes to the zero-order character of the kinetic release.

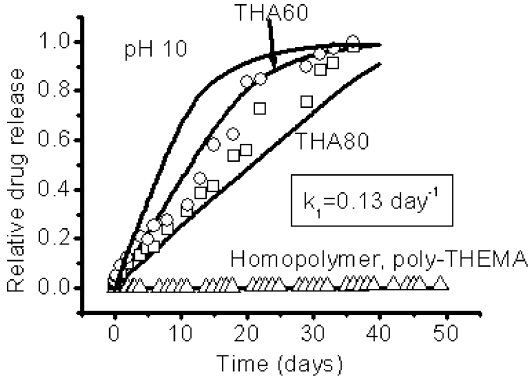


Figure 9. Release profiles of Triflusal from polyTHEMA and THEMA−AMPS copolymers at pH 10

The Triflusal release from the homopolymer polyTHEMA, which is comparatively negligible, has been also plotted. This case corresponds to a polymer with only TTT triads, and for that reason, no release is expected by this model.

In conclusion, this kinetic model, based on microstructural arrangement of hydrophilic and hydrophobic sequences, fits the experimental drug release data of the hydrophilic/hydrophobic THEMA−AMPS copolymers taken as model system. The good agreement indicates the great relevance of the polymer microstructure for such a heterogeneous copolymer in the physiological behavior.

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