Hydrophilic Drug Release from Bioerodible Polyanhydride Microspheres

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ABSTRACT: The present investigation was carried out to develop bioerodible drug delivery systems. Copolymers of fumaric anhydride and isophthalic anhydride were synthesized by melt polycondensation. To synthesize a copolymer with known composition, soluble in common organic solvents, a prepolymer of each monomer was first prepared. Copolymers were synthesized by mixing two prepolymers followed by melt polycondensation of the resulting mixture with a specific ratio of each prepolymer. Microspheres loaded with theophylline and diltiazem hydrochloride (DHC) were obtained using the solvent removal method in an oil-in -oil (O/O) emulsion system. The size of the drug loaded microspheres was less than 75 μm, which is suitable for subcutaneous or intramuscular injection. DHC was incorporated in a polymeric carrier better than theophylline because of its solubility in chloroform and dichloromethane. *In vitro* release of two drugs in the phosphate buffer solution indicated that the release profile of DHC was closer to a zero-order kinetic profile compared with theophylline. Finally, drug release data was compared with three semiempirical models. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 83: 1457–1464, 2002

Key words: drug delivery systems; isophtalic anhydride; fumaric anhydride; theophylline; diltiazem hydrochloride

INTRODUCTION

The emergence of recombinant DNA technologies, which has created a variety of proteins and other substances that have potential as therapeutic agents in the treatment of diseases, and the advances of medical and pharmaceutical sciences necessitate the development of novel drug delivery systems.

After sustained release systems, controlled release systems were developed in which drugs were embedded in a piece of plastic or polymer, or placed in a solution in a pump. In these systems drug release rates are determined almost exclusively by the design of the polymeric system or pump.

Biodegradable controlled release systems have an advantage over other controlled release systems in obviating the need to surgically remove the drug-depleted device.[1,2] Potentially, biodegradable matrix systems also enjoy a number of other advantages in terms of simplicity in design and predictability of release if release is controlled solely by the degradation of the matrix.[3,4] In many cases, however, the release is augmented by diffusion through the matrix, rendering the process difficult to control, particularly, if the matrix is hydrophilic and thereby absorbs water,

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promoting degradation in the interior of the matrix. Achieving a heterogeneous degradation requires the rate of hydrolytic degradation on the surface to be much faster than the rate of water penetration into the bulk. The ideal polymer would have a hydrophobic backbone, but with a water labile linkage.

In designing any biodegradable system that would erode in a controlled heterogeneous manner without requiring any additives, polyanhydrides due to high liability of their anhydride linkage may be promising candidates.[5]

Aromatic polyanhydrides were synthesized in 1909 by Bucher and Slade.[6] Aliphatic polyanhydrides were initially proposed by Hill and Carothers in 1930 to be a substitute for polyester textile applications.[7] The idea was later rejected because of their hydrolytic instability. It is this property, however, that renders polyanhydrides appealing for controlled release applications. The hydrophilic anhydride linkage provides the basis for using a variety of backbones and yet ensuring biodegradation.

Polyanhydrides are a well-studied class of bioerodible polymers. Polyanhydrides of aromatic diacids offer several advantages over aliphatic polymers. They possess longer release and degradation times when used as degradable materials for drug release,[8] possess high thermodynamic stability in solid state and in organic solutions,[9] and are more favorable with respect to drug polymer interactions, especially for proteins.[10] Controlled release implants from polyanhydrides are being developed for the treatment of osteomyelitis.[11]

Unfortunately, aromatic polyanhydrides have in general low solubility in common organic solvents (less than 0.1% chlorinated, aromatic, or aliphatic hydrocarbons), and have high melting points. Neither of these properties limit uses of aromatic polymers because they cannot be fabricated by either solvent technique due to their low solubility, nor using melt processing techniques due to their high melting points. Aromatic polymers are usually highly crystalline, and such polymers are characterized by their brittleness and poor flexibility.[12]

One way to overcome these limitations is copolymerization of aromatic diacids with aliphatic diacids. The resulting copolymers have relatively low melting points and increased mechanical strength as the aromatic content increased. However, these copolymers containing more than 65% aromatic are insoluble in common organic solvents and have high crystallinity and melting points.

The ideal polyanhydride would be one that possesses the properties of an aromatic polymer, good hydrolytic and thermodynamic stability, and superior mechanical strength, yet is soluble in common organic solvents and melts at temperature below 200°C.

Isophthalic acid is an aromatic diacid and fumaric acid is an unsaturated linear diacid. But fumaric acid was included among the aromatic monomers because of its similar properties to aromatic polymers. Therefore, polyanhydride prepared using these diacids is fully aromatic, yet melts at temperatures below 100°C and is very soluble in dichloromethane or chloroform (greater than 15%w/v). This was achieved by adding a second aromatic diacid to the copolymer composition. The second aromatic diacid introduces irregularity in the polymer chains that dramatically changes the polymer properties, the melting points, and solubility in the common solvents such as dichloromethne or chloroform.

Solubility of polymers is a major factor on their uses and applications. The most commonly used organic solvents for controlled release fabrications are dichloromethane and chloroform. These are volatile, nonflammable, and inexpensive solvents that can be completely removed from the product by vacuum.

The main objective of the present study was to develop bioerodible drug delivery systems for zero-order drug release. Therefore, it was decided to synthesize polyanhydride copolymer with high aromatic content, of at least 70% aromatic diacid units, which is soluble in organic solvents such as dichloromethane or chloroform, melts at temperatures below 200°C, and has low crystallinity.

In this study poly (fumaric anhydride-*co*-isophthalic anhydride) was synthesized by melt polycondensation and the resulting polymer was formulated into microspheres loaded with a sparggingly water soluble drug such as theophylline and a water-soluble drug such as DHC using a solvent extraction technique. The important parameters of this microencapsulation process are the partition of the organic solvent in oil, the transport rate of this partitioning, the surface-to-volume ratio of the dispersed phase, and the rate of hardening of the polymer in the presence of the drug particles. Then *in vitro* release experiments of two drugs were carried out. Finally, drug release data was compared with three semiempirical models—

the Ritger model, the Potowinski model, and the zero-order kinetic model.

MODELING OF DRUG RELEASE

Modeling of the controlled release of drugs from polymeric devices has been the subject of much research in the past 20 years. Most of the presented models have been based on Fickian diffusion, published by Crank.[13] Regarding pharmacology, other equations, like the Higuchi equation,[14] have been acceptably presented for drug delivery from tablets.

In this study the semiempirical Ritger's model,[15] zero-order kinetic model, and semiempirical Potowinski's model[16] have been used for predicting release behavior of theophylline and DHC from poly(isophthalic-*co*-fumaric) anhydride. These models are simple, and can define kinetics of drug release from prepared devices. Thus, they are simple and useful tools to predict the results and kinetics of the release data. However, they do not involve geometrical and physical characteristics of the given device and it is a major limitation of these models.

For a slab in isothermal condition and one-dimensional diffusion Ritger solved Fickian diffusion equation to predict drug release from unswellable devices; and has obtained fractional release (M_t/M_∞), in the form of an error function series. In regarded to the resulting series, it is obvious that, at short periods, fractional release is proportional to the square root of time:

$$(M_t/M_\infty) = 4 \cdot [D \cdot t/\pi \cdot I^2]^{1/2} \quad (1)$$

Here, I is the thickness of slab and D is diffusivity. In this case, a short period approximation is valid for totally 60% of release process.

In the second limiting case, drug delivery is not depended on time, which means drug release follows zero-order kinetics. This situation is presented by the following equation:

$$(M_t/M_\infty) = k \cdot t \quad (2)$$

The above equations can be written as the following general form:

$$(M_t/M_\infty) = k \cdot t^n \quad (3)$$

where the k constant depends on the characteristics of polymer matrix and drug, and n is the diffusional exponent, which defines diffusion mechanism.

Equation (3), also is valid for the first 60% of the fractional release. For a slab, Fickian diffusion is defined by $n = 0.5$, and non-Fickian diffusion is presented by $n > 0.5$.

The solution of the Fickian diffusion equation in spherical and cylindrical geometries and comparison of the results with eq. (3) show semiempirical eq. (3) with $n = 0.5$ valid only for the first 10–15% of total release. This problem can be solved by definition of one new diffusional limit for n, based on the first 60% of fractional release for any geometrical shape. In this basis, Fickian diffusion from a cylinder and a sphere is presented by eq. (3) with $n = 0.45$, and $n = 0.43$, respectively. Thus, for a sphere, drug release follows non-Fickian diffusion, when $0.43 < n < 1$. As mentioned above, eq. (3) is only valid for the first 60% of the total release. Hence, Potowinski has presented the following equation:

$$(M_t/M_\infty) = 1 - \exp \cdot [-k(t + b)] \quad (4)$$

The presence of constant b in this equation makes it suitable for use in systems where the drug is partially located on the device surface ($b > 0$), and also for systems with erodible films, whose removal is followed by active agent release ($b < 0$). Potowinski's studies showed that eq. (4) can be used for prediction of total release profile.

EXPERIMENTAL

Polymer Synthesis

The poly(fumaric anhydride-*co*-isophthalic anhydride) was synthesized by melt polycondensation following the method described by Domb.[17] In this method isophthalic acid and fumaric acid were first purified, then prepolymers of two purified monomers were prepared. Prepolymers of two dicarboxylic acids were prepared separately by reacting them with acetic anhydride. Then, these prepolymers were purified. Finally, prepolymers underwent melt polycondensation in a 2 × 20-cm glass tube with a side arm and equipped with a capillary inlet. The tube was immersed in an oil bath at 180°C. After 1 min, when prepolymers were melted, high vacuum (greater than 100 mmHg) was applied through the side arm.

The crude polymer was purified by precipitation in dry petroleum ether from a dichloromethane solution. The precipitate was then extracted with anhydrous ether for several hours at room temperature. When 2 mol % cadmium acetate dehydrate was used as a catalyst, it was mixed with the prepolymers prior to polymerization and then was removed from polymer solution by filtration.

Microsphere Preparation

Microspheres were prepared from polymer solution of a polymer–drug mixture as follows: 1 g polymer was dissolved in 10 mL methylene chloride, and the drug was suspended in the solution, mixed, dropped into silicon oil containing 1 to 5% of span 80 using a glass syringe with a 22 G stainless steel needle, and stirred at a known stirring rate (400–800 rpm) in a round bottom vessel. After 1 h, petroleum ether was introduced to the medium and stirring was continued for another hour. The microspheres were isolated by filtration, washed with petroleum ether, freeze dried over night, and stored in desicator within a freezer.[18] The particle size of microspheres was determined by scanning electron microscopy (SEM, S-360 Cambridge).

The entrapment of DHC in the polymer matrix was measured by dissolution of the loaded microspheres in chloromethane, mixing it with distilled water and finally measuring concentration of the drug in an aqueous phase. The extraction efficiency after three stages was 95.0%. Theophylline is not soluble in dichloromethane, and after dissolution of loaded microspheres in dichloromethane, theophylline was removed from solution by filtration and weighted.

In Vitro Release

The experiments for drug release measurements from 5% w/w loaded microspheres with theophylline and or DHC in 0.1 *M* phosphate buffer solution (pH = 7) were carried out in a water bath at 37°C equipped with a shaker.

In these tests, 0.04 g of drug loaded microspheres were added to 40 mL of 0.1 *M* phosphate buffer solution (pH = 7) in a flask. The flask was closed to prevent evaporation and probable changes of pH. This flask was placed in a 37°C bath equipped with a shaker that was kept operating all the time. At specific time intervals, 1 mL of the solution was removed and replaced with fresh phosphate buffer. The concentration of released drugs for theophylline, and DHC were measured by a spectrophotometer at 270 and 240 nm, respectively.

RESULTS AND DISCUSSION

Polymer Characterization

The weight-average molecular weight, *Mw*, of the synthesized poly(isophthalic anhydride-*co*-fumaric anhydride) 75 : 25, determined by GPC (150-C ALC/GPC model Waters system), was 17,000.

In polymer synthesis, cadmium acetate dehydrate was used as a catalyst. This material is toxic, but as mentioned by Domb,[17] it is insoluble in dichloromethane and thus was removed completely from the polymer solution by filtration.

The solubility of the polymer has been tested in dichloromethane, chloroform, tetrahydrofuran (THF), and toluene. These tests show that the polymer was very soluble in dichloromethane and chloroform (greater than 15%w/v), and insoluble in THF and toluene. Thus, this polymer is suitable for fabrication of drug delivery systems by solvent methods.

As mentioned earlier, polyanhydrides prepared from the very pure, isolated prepolymers are specially useful for biomedical applications such as controlled release of drugs because of the agreement between calculated and actual composition of the polymer. This agreement permits appropriate tailoring of the chemical composition and thereby, hydrophilicity and hydrophobicity of the resulting polymers. Also, extensive toxicology information on a range of polyanhydrides is available, which indicate the most polyanhydrides prepared from isolated pure prepolymers are biocompatible.[19] The main point is a method of synthesis of highly pure anhydride copolymer of known composition wherein the key element is the use of individually prepared pure prepolymers.

For this purpose, the individual prepolymers were mixed together and polymerized to form a copolymer. Calculated composition of the resulting copolymer was 75 mol % of isophthalic acid and actual composition, determined by ^{1}H NMR analysis (Bruker spectrometer), was 76.8 mol % of isophthalic acid. The ratio of IPA and FA in poly(IPA-*co*-FA) was determined from peak integration at 7.4–8.6 ppm (IPA) and 6.9 ppm (FA). This result indicated isolated prepolymers for the synthesis of polyanhydride was very pure. Thus,

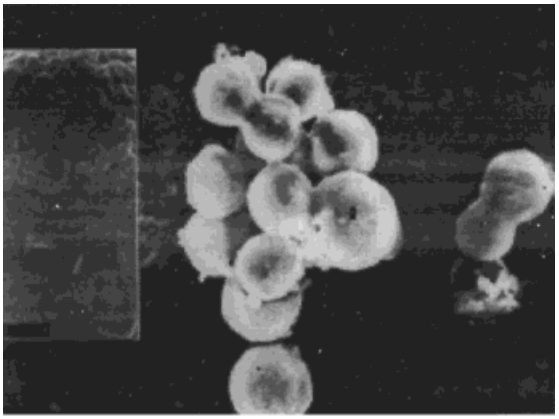
making them suitable for preparation of drug delivery systems.

Microparticles

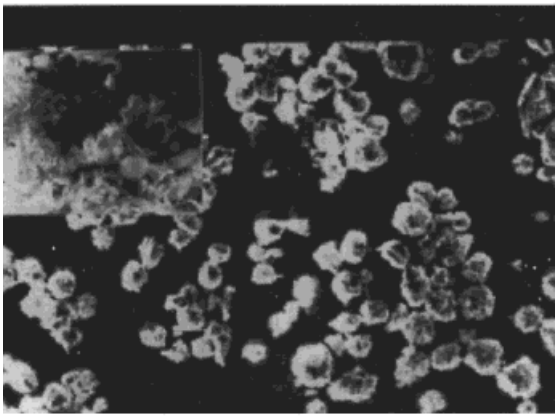
The polyanhydride with composition above 70% aromatic unit, synthesized in this study, was very soluble in chloroform and dichloromethane. Thus, it was suitable for fabrication of microspheres using an O/O emulsion with solvent-removal method. The advantage of the method of drug microencapsulation is that the preparation carried out in organic solvent prevents the hydrolytic degradation of the polymer and dissolution of any water-soluble drug. The particle size of the resulting microspheres was determined by scanning electron microscopy (SEM). Two types of surfactants, used for preparation of microspheres, were Span 80 and lecithin. When Span 80 was used as the surfactant, the resulting microparticles had a spherical shape [Fig. 1(a)], but when lecithin was used as the surfactant, the resulting particles were not precisely spherical [Fig. 1(b)]. Thus, the rest of the experiments for microspheres preparation were carried out using Span 80 as the surfactant. The polyanhydride microspheres loaded with 5% (w/w) (drug/polymer) theophylline and or DHC are shown in Figure 2. All the resulting particles were spherical, and had a dense and nonporous surface with few microcracks on them.

The size of the resulting microspheres was less than 75 μm, which is suitable for subcutaneous or intramuscular injection. This result is comparable with those obtained by Mathiowitz.[18] Adding Span 80 to silicon oil resulted in a binary system consisting of large droplets, immediately after adding the polymer–drug mixture to the surfactant–silicon oil solution. There were also many surfactant droplets devoid of microspheres. The resulting microparticles, prepared in the absence of Span 80, did not have regular shape (Fig. 3).

The series of steps leading to the microsphere formation can be described as follows. Stirring disrupted the dichloromethane droplet coming in contact with the silicon oil. Simultaneously, dichloromethane started partitioning into the oil phase. At some point, solidification took place and the drug particles were trapped in the microspheres. The surfactant probably serves two important functions in regulating the microsphere formation. In engulfing the solution droplets, the surfactant acts as a transport barrier for gradual extraction of the solvent. The surfactant layer might also prevent aggregation of the solidified microspheres.



(a)



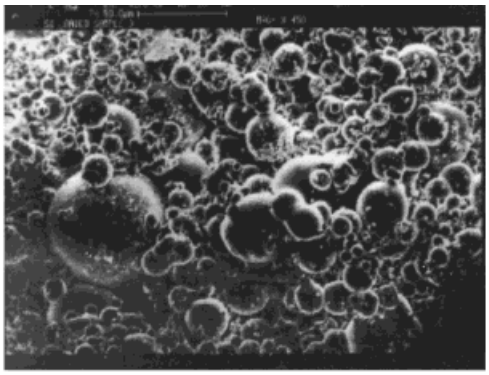
(b)

Figure 1 SEM of polyanhydride microspheres prepared in the presence of (a) 1% Span 80 as the surfacant; and (b) 5% Lecithin as the surfacant.

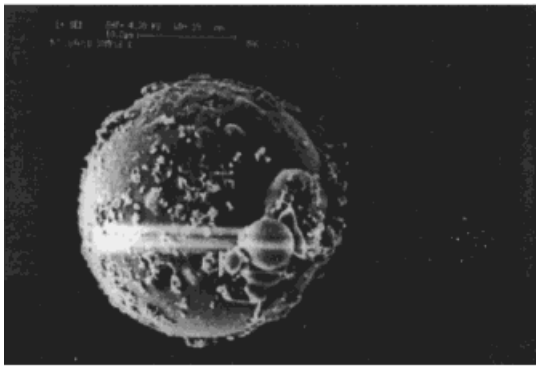
In Vitro Release

The release profile for two drugs, theophylline and DHC, vs. time is presented in Figure 4. It can be seen that both drugs were released within 100 h. No lag time for drug release was found, as was also observed for poly(lactic acid) and poly(lactic acid-*co*-glycolic acid), which are known to undergo bulk erosion.[20]

A considerable amount of theophylline was released during the first hours of the incubation.



(a)



(b)

Figure 2 SEM of polyanhydride microspheres: (a) loaded with 5% DHC; (b) loaded with 5% theophylline.

This is proof for deposition of theophylline on the surface of the microspheres due to improper loading, resulting from incompatibility of the drug and polymer matrix and low solubility of theophylline in dichloromethane. Theophylline deposition on the surface of microspheres also confirms the scanning electron microscopy observation shown in Figure 2.

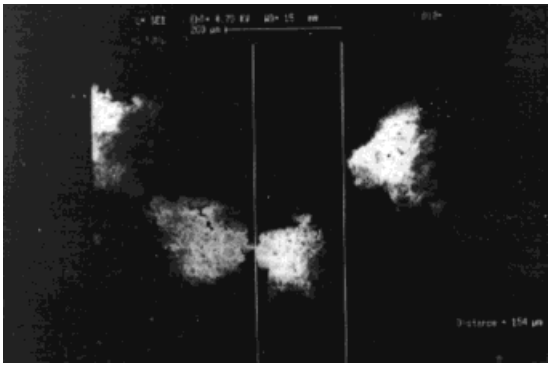


Figure 3 SEM of polyanhydride microparticles prepared in the absence of a surfacant.

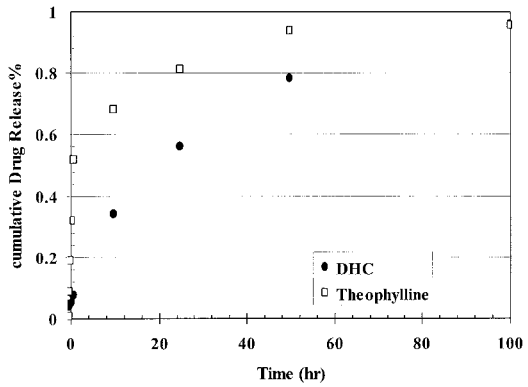


Figure 4 *In vitro* drug release profile from polyanhydride microspheres.

DHC release from loaded microspheres showed that approximately one-third of the loaded drug was released at one-tenth (10 h) of the delivery process time. This result can be attributed to a higher concentration of DHC in the outer layers of the microsphere.

Prediction of Release Data

The predictions of different kinetic models for the release of DHC and theophylline are compared with experimental data in Figures 5 and 6, respectively. The corresponding correlation coefficients for fitting experimental results with different kinetic models are summarized in Table I.

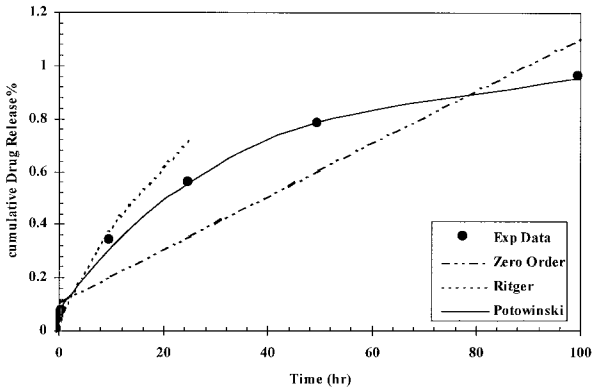


Figure 5 Comparison of semiempirical kinetics models with experimental results for DHC release from polyanhydride microspheres.

These results indicate that Ritger's semiempirical model, with $n = 0.99$, had good agreement with experimental data for DHC release. This quantity of n indicates that drug release was controlled by non-Fickian diffusion. As mentioned before, this model is valid only for the first 60% of total release, and this has limited its application. The Potowinski model agreed better than two other models with the experimental data, for a total release profile. Thus, it can be used for modeling of this device. In addition, the resulting b constant of this model was 2.79 ($b > 0$), indicating that drug is partially located on the surface of microspheres, which is consistent with SEM observation.

Considering the results of Figure 6 and Table I, none of the semiempirical models are suitable for the complete prediction of theophylline release from this drug delivery system, but predictions of Potowinski's model is close the experimental results.

CONCLUSION

Polynahydride copolymers containing 75 mol % of the aromatic unit was synthesized by melt polycondensation of purified prepolymer mixtures. This method of synthesis resulted in a copolymer, where its calculated composition was approximately equal with actual composition, and it was very soluble in chloroform and dichloromethane, and melted at temperatures below 200°C.

Biodegradable polyanhydride microparticles, loaded with DHC and theophylline, were prepared by an O/O solvent removal method. The microspheres were spherical in shape with a dense structure at the outer surface. Both DHC and theophylline were released completely at 100 h. The entrapment of DHC and theophylline in the polymer matrix was 95.4 and 94.8% of the initial amount of drugs used for the polymer loading, respectively. Finally, it can be concluded that the Potowinski model has good and sufficient agreement with the experimental data of DHC and theophylline release, respectively.

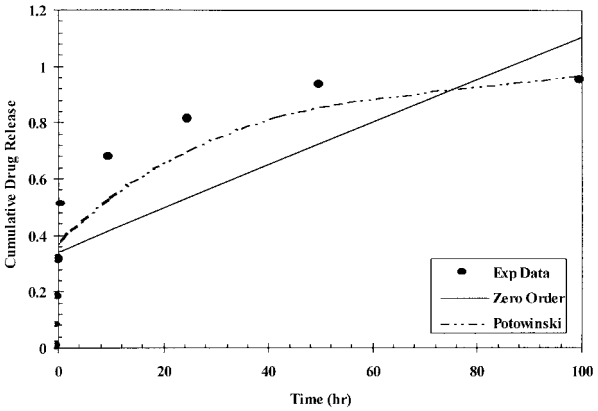


Figure 6 Comparison of semiempirical kinetics models with experimental results for theophylline release from polyanhydride microspheres.

Table I Comparison of Semiempirical Models with Experimental Data of Drug Release

Drug	Model (Kinetic)	Correlation Coefficient
DHC	Zero-order	0.9344
	Ritger	0.9896
	Potowinski	0.9926
Theophylline	Zero-order	0.7365
	Ritger	Cannot be used
	Potowinski	0.9171

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LIST OF SYMBOLS AND ABBREVIATIONS

b	Constant of Potowinski's equation
D	Diffusivity
DHC	Diltiazem hydrochloride
FA	Fumaric acid
GPC	Gel permeation chromatography
IPA	Isophtalic acid
k	System parameter that depend on the nature of the polymer/active agent interaction
I	Thickness of slab
M_t	Amount of active agent released by time t
M_∞	Final released amount of active agent
n	Diffusional exponent
O/O	Oil-in-oil
rpm	Revolution per minute
SEM	Scanning electron microscopy
t	Time
THF	Tetrahydrofuran

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