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European Journal of Pharmaceutics and Biopharmaceutics 56 (2003) 271–279
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Mathematical modeling of drug release from bioerodible microparticles: effect of gamma-irradiation

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Received 7 April 2003; accepted in revised form 4 June 2003

Abstract

Bioerodible polymers used in controlled drug delivery systems, such as poly(lactic-co-glycolic acid) (PLGA) undergo radiolytic degradation during γ-irradiation. In spite of the considerable practical importance, yet only little knowledge is available on the consequences of this sterilization method on the resulting drug release patterns in a quantitative way. The major objectives of the present study were: (i) to monitor the effects of different γ-irradiation doses on the physicochemical properties of drug-free and drug-loaded, PLGA-based microparticles; (ii) to analyze the obtained experimental results using adequate mathematical models; (iii) to get further insight into the occurring physical and chemical phenomena; and (iv) to relate the applied γ-irradiation dose in a quantitative way to the resulting drug release rate. 5-Fluorouracil-loaded, PLGA-based microparticles were prepared with an oil-in-water solvent extraction method and exposed to γ-irradiation doses ranging from 0 to 33 kGy. Size exclusion chromatography, differential scanning calorimetry, scanning electron microscopy, particle size analysis, determination of the actual drug loading and in vitro drug release kinetics were used to study the effects of the γ-irradiation dose on the physicochemical properties of the microparticles. Two mathematical models—a simplified and a more comprehensive one—were used to analyze the experimental results. The simplified model considers drug diffusion based on Fick's second law for spherical geometry and a Higuchi-like pseudo-steady-state approach. The complex model combines Monte Carlo simulations (describing polymer erosion) with partial differential equations quantifying drug diffusion with time-, position- and direction-dependent diffusivities. Interestingly, exponential relationships between the γ-irradiation dose and the initial drug diffusivity within the microparticles could be established. Based on this knowledge both models were used to predict the resulting drug release kinetics as a function of the γ-irradiation dose. Importantly, the theoretical predictions were confirmed by experimental results.

Keywords: Microparticle; γ-Irradiation; Modeling; Bioerosion; PLGA

1. Introduction

Bioerodible, polymeric microparticles offer various advantages compared to conventional dosage forms, such as the possibility to control the resulting drug release rates accurately over prolonged periods of time, easiness of administration, good biocompatibility and complete bioerosion (avoiding the removal of empty remnants). Consequently, the practical importance of these advanced drug delivery systems is remarkably increasing. When administered parenterally, γ-irradiation is currently the method of choice for the terminal sterilization of this type of delivery system [1,2]. Ethylene oxide is not suitable because of the toxicity of its residues, and exposure to dry or moist heat is not possible due to the thermosensitivity of these devices.

It is well known that PLGA undergoes radiolytic degradation during γ-irradiation [1–4]. Due to the resulting decrease in the average polymer molecular weight, the release rate of an incorporated drug can significantly be affected. Generally, the drug release rate increases with increasing irradiation dose. Kissel and co-workers thoroughly studied the effects of γ-irradiation on the physicochemical properties of drug-free and drug-loaded, PLGA-based microparticles [1,2,4]. For example, electron

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doi:10.1016/S0939-6411(03)00104-8

paramagnetic resonance spectroscopy, gel permeation chromatography, differential scanning calorimetry, gas chromatography–mass spectroscopy and scanning electron microscopy were used to investigate the influence of γ-irradiation on radical formation, polymer degradation and other system characteristics. Interestingly, the polydispersity indices were almost unaffected by the irradiation process, indicating that the induced radiolytic chain cleavage is random and not preferably occurring at the end-groups of the polymer chains. Importantly, certain drugs interacted with the bioerodible polymer via radical formation during γ-irradiation, e.g. captopril was found to be partially oxidized to its disulfide and probably coupled to the PLGA [1]. In contrast, the actual loading of tetracycline HCl-containing microparticles was not affected by this sterilization method [4]. Recently, Montanari et al. [5] suggested that clonazepam has a radio-stabilizing effect on the polymer matrix in PLGA-based microparticles. Hausberger et al. [3] studied the effect of the applied γ-irradiation dose on the decrease in polymer molecular weight, onset time for mass loss upon exposure to the release medium and zero order mass loss rate in/of PLGA-based microparticles. Interestingly, the latter was independent of the irradiation dose, whereas the molecular weight and onset time for mass loss decreased with increasing irradiation dose.

In spite of the considerable practical importance, yet only little knowledge is available on the consequences of γ-irradiation on the resulting drug release patterns in a quantitative way [6,7]. This might be attributable to the fact that the mathematical modeling of drug release from bioerodible delivery systems is rather complex [8]. In addition to physical mass transport phenomena, chemical reactions have to be considered. The latter change the conditions for the mass transfer processes continuously, rendering the mathematical treatment difficult [9,10]. To simulate polymer degradation and erosion either empirical or diffusion and chemical reaction-based models can be applied. A subclass of the latter simulates polymer degradation as a random event using direct Monte Carlo techniques [11,12]. The significant advantage of these models compared to empirical approaches is that they are physicochemically more realistic. Recently, we proposed a new simplified and a novel more complex mathematical model to describe drug release from bioerodible microparticles [13,14]. The simplified model considers drug diffusion based on Fick's second law for spherical geometry, a Higuchi-like pseudo-steady-state approach and the dependence of the drug diffusivity on the polymer molecular weight. The complex model combines Monte Carlo simulations (describing polymer erosion) with partial differential equations quantifying drug diffusion with time-, position- and direction-dependent diffusivities. In contrast to the simplified approach, this model is also applicable to tri-phasic drug release behavior.

In the present study the effects of γ-irradiation on the release kinetics of the anticancer drug 5-fluorouracil (5-FU) from PLGA-based microparticles were studied. These devices are used for the treatment of brain tumors [15,16]. Malignant gliomas represent 13–22% of the brain cancers. Regardless of the treatment method the median survival time is less than 1 year. Despite surgery, external beam radiation therapy and systemic chemotherapy, these tumors tend to recur within centimeters of their original location. To decrease the risk of local recurrences, anticancer drug-loaded, bioerodible microparticles can be injected into the wall of the resection cavity of the tumor. First clinical trials with 5-FU-loaded PLGA microparticles showed promising results [17].

The major aims of this work included: (i) to study the effects of different γ-irradiation doses on the physicochemical properties of drug-free and 5-fluorouracil-loaded, PLGA-based microparticles as well as on PLGA powder; (ii) to analyze the experimental results using adequate mathematical models; (iii) to better understand the underlying drug release mechanisms; and (iv) to establish a quantitative relationship between the applied γ-irradiation dose and the resulting drug release rate.

2. Materials

Poly(D,L lactic-co-glycolic acid) (PLGA; Resomer RG 506; PLGA 50:50; containing 25% D-lactic units, 25% L-lactic units and 50% glycolic units) and 5-fluorouracil (5-FU) were obtained from Boehringer Ingelheim (Paris, France) and Roche (Neuilly sur Seine, France), respectively.

3. Experimental methods

3.1. Microparticle preparation

5-FU-loaded, PLGA-based microparticles were prepared with an oil-in-water (O/W) solvent extraction technique. A dispersion of 4 g drug within 45 ml dichloromethane was prepared using an ultra turrax (13,500 rpm, 4 min) (Ika, T25 basic/S25N-10G, Staufen, Germany). The PLGA (5 g) was added to this dispersion, which was subsequently stirred to allow complete polymer dissolution. This organic phase was emulsified into 1500 ml aqueous polyvinyl alcohol solution (10% w/w). The resulting dispersion was stirred with a propeller at 375 rpm. The addition of 4500 ml water under stirring allowed microparticle hardening. The latter were separated by filtration under nitrogen pressure (0.8 bar, filtration system supplied by Sartorius, Palaiseau, France) with a cellulose ester filter membrane (8 μm, Millipore, Saint Quentin en Yvelines, France), freeze-dried and sieved (125 μm). To minimize the amount of residual organic solvent the microparticles were also vacuum dried at 37 °C for 72 h. Drug-free microparticles were prepared accordingly, dissolving the PLGA in pure dichloromethane.

Table 1
Effect of the applied γ-irradiation dose on the mean diameter of drug-free and 5-FU-loaded, PLGA-based microparticles

Drug-free microparticles		5-FU-loaded microparticles	
γ-Irradiation dose (kGy)	Diameter (μm)	γ-Irradiation dose (kGy)	Diameter (μm)
4.3	33	4.4	57
10.6	31	10.5	59
17.3	32	17.0	57
23.2	30	22.9	58
27.6	31	28.0	58
32.5	30	33.1	59

3.2. γ-Irradiation

Approximately 700 mg drug-loaded or drug-free microparticles or pure polymer powder were placed in vials, sealed under vacuum and exposed (under cooling) to different γ-irradiation doses (ranging from 4 to 33 kGy, Table 1) using a ^{60}Co source (performed at Ionosos, Dagneux, France).

3.3. Determination of drug loading

The actual drug loading was determined by dissolving accurately weighed amounts of microparticles (approx. 7 mg) in 50 ml dimethylsulfoxide, and subsequent UV drug detection at $\lambda = 266$ nm (Uvikon 922, Kontron, St Quentin en Yvelines, France).

3.4. In vitro drug release studies

In vitro drug release was determined by placing microparticles (approx. 40 mg) within dialysis bags (Spectra/Por membrane, molecular weight cut-off 6–8 kDa; Bioblock, Illkirch, France) at the bottom of USP XXV paddle apparatus glass vessels (Sotax AT7, Sotax, Basel, Switzerland). Phosphate buffer pH 7.4 (containing 0.1% sodium azide to avoid microbial growth) (7 ml in each dialysis bag, plus additional 500 ml in each vessel) was chosen as release medium, kept constant at 37 °C and stirred at 100 rpm. The apparatus was protected from light. At predetermined time intervals, 1-ml samples were withdrawn and analyzed UV-spectrophotometrically (Uvikon 922; $\lambda = 266$ nm). Each experiment was conducted in triplicate.

3.5. Particle size analysis

The mean diameter of drug-free and 5-FU-loaded, PLGA-based microparticles was measured with a Coulter Counter (Multisizer, Coultronics, Margency, France), suspending the particles (approx. 10 mg) in an aqueous Tween 80 solution, and diluting with Isoton II (Coultronics, Margency, France).

3.6. Differential scanning calorimetry

The glass transition temperature (T_g) of the PLGA in drug-free and 5-FU-loaded microparticles as well as in the form of free polymer powder was determined by differential scanning calorimetry (DSC, Mettler Toledo, Viroflay, France). Approximately 5-mg samples were heated in sealed aluminum pans (investigated temperature range: −30 to +90 °C, heating rate: 10 °C/min, two heating cycles).

3.7. Size exclusion chromatography

Drug-free, 5-FU-loaded microparticles or pure polymer powder were dissolved in dimethylsulfoxide (0.5% w/v). One volume part of this solution was mixed with three volume parts of the mobile phase (tetrahydrofurane/methanol/acetic acid, 85:15:0.8, by vol.). Approximately 200 μl of this mixture were injected into a size exclusion (gel permeation) chromatography apparatus equipped with a precolumn (Shodex KGF, Waters, Saint Quentin en Yvelines, France), two main columns (Styragel HR1, Waters; PL-gel 5μm 10^E 4A, Polymer Laboratories, Marseille, France) and a refractometric detector (RID-10A, Shimadzu, Touzart et Matignon, Courtaboeuf, France). All measurements were performed at a flow rate of 1 ml/min at room temperature. The system was calibrated with polystyrene standards (PS-2, Polymer Laboratories, Marseille, France). All indicated molecular weights are weight average molecular weights (M_w).

3.8. Scanning electron microscopy

The morphology of 5-FU-loaded microparticles was characterized by scanning electron microscopy (SEM). Samples were carbon coated (10 nm) using a MED 020 (Baltec, Balzers, Liechtenstein) and observed on a JEOL 6301F field emission microscope (JEOL, Paris, France) (voltage: 5 kV).

4. Theoretical methods

4.1. Simplified mathematical model

The simplified mathematical model is based on the assumption of linear, pseudo-steady state drug concentration gradients established within the microparticles upon water imbibition due to the high 'initial drug loading/drug solubility' ratio [13]. Higuchi derived the well known relationship between the relative amount of drug released and the square root of time under these conditions for the case of planar devices [18]. Later, he extended his model also to spherical geometry, deriving an implicit mathematical equation [19]. A similar approach was used by Koizumi and Panomsuk [20] leading to an (approximate)

explicit solution describing drug release from non-erodible, spherical devices having the advantage to be easier to handle than the respective equation derived by Higuchi:

$$M_t = 4\pi r^2\left[\sqrt{2(c_0 - c_s)c_s Dt} + \frac{4c_s Dt}{9r}\left(\frac{c_s}{2c_0 - c_s} - 3\right)\right] \quad (1)$$

Here, M_t is the cumulative absolute amount of drug released at time t; r represents the radius of the spherical device; c_0 and c_s are the initial drug concentration and the solubility of the drug within the system, respectively; and D denotes the constant diffusion coefficient of the drug.

Recently, this approximate pseudo-steady state solution was combined with an equation taking into account the dependence of the drug diffusion coefficient on the decreasing average polymer molecular weight (M_w) in bioerodible drug delivery systems [13]:

$$D(M_w) = D_0 + \frac{k}{M_w} \quad (2)$$

where D_0 is the diffusion coefficient of the drug in the non-degraded polymer ($t = 0$), and k is a constant. Considering pseudo first order polymer degradation kinetics upon contact with the release medium, the decrease in polymer molecular weight was calculated as follows:

$$M_w(t) = 78.4exp\left(-k_{degr}t\right) \quad (3)$$

where k_{degr} is the pseudo first order degradation rate constant of the polymer.

Eqs. (1–3) were fitted to experimentally determined 5-FU release rates from PLGA-based microparticles (which had been exposed to different γ-irradiation doses: 4.4, 10.5, 17.0, 22.9, 28.0, and 33.1 kGy) [6]. To be able to evaluate the predictive power of the mathematical model, one γ-irradiation dose was arbitrarily selected (4.4 kGy) and the respective experimental results excluded from the fitting procedure. Based on the system-specific parameters determined with microparticles exposed to the other γ-irradiation doses a quantitative relationship between the applied dose and the initial drug diffusion coefficient (D_0) was established. Using this relationship and the mathematical model the resulting in vitro drug release kinetics from microparticles exposed to 4.4 kGy were theoretically predicted and compared to the experimental results (it has to be pointed out that these data were, thus, not completely independent from the data used for the determination of the system-specific parameters). For the implementation of the mathematical model the programming language C++ was used (Borland C++ 6.0).

4.2. Comprehensive mathematical model

The complex mathematical model considers polymer degradation and drug diffusion in a physically more realistic way than the simplified approach does. The random process of polymer chain cleavage upon water imbibition is simulated using a Monte Carlo technique. The spherical microparticles are divided into approximately 10 000 concentric rings of equal volume. At $t = 0$ (before exposure to the release medium) each ring represents either drug or non-degraded polymer. Due to the identical volume of the polymer rings it is assumed that each of them contains a similar number of cleavable ester bonds. Thus, the probability with which a ring representing non-degraded polymer degrades upon its first contact with water is similar for all rings. As the ester bond cleavage is a random process, not all polymer rings degrade exactly after the same time period upon contact with water. They possess individual, randomly distributed 'lifetimes'. For reasons of simplicity, polymer degradation and the subsequent diffusion of the degradation products out of the device are simulated as one event (polymer erosion). As soon as a polymer ring comes into contact with water, its 'lifetime' starts to decrease. After the latter has expired, the ring is assumed to erode instantaneously. The 'lifetime', t_{lifetime}, of a ring is calculated as a function of the random variable ϵ (integer between 0 and 99):

$$t_{lifetime} = t_{average} + \frac{(-1)^{\epsilon}}{\lambda} ln\left(1 - \frac{\epsilon}{100}\right) \quad (4)$$

where t_{average} is the average 'lifetime' of the non-degraded polymer rings upon contact with water, and λ is a constant (being characteristic for the type and physical state of the polymer).

Using this Monte Carlo technique, the time-dependent changes in the inner and outer structure of the polymeric network of the bioerodible microparticles can be simulated. Knowing the status of each ring ('non-eroded polymer' or 'pore') at each time point, the porosities in radial and axial direction (depending on time and position) can be calculated (increasing numbers of pores lead to increasing porosities). It is important to consider the dependence of the porosity on the direction (axial/radial) because the inner structure of the microparticles is heterogeneous. Based on the porosity values, the position- and direction-dependent drug diffusivities within the systems can be calculated as a function of the exposure time to the release medium (increasing porosities lead to increasing diffusivities). This information is essential for the accurate calculation of the diffusional mass transport processes using Fick's second law [21]:

$$\frac{\partial c}{\partial t} = \frac{1}{r}\left\{\frac{\partial}{\partial r}\left(rD\frac{\partial c}{\partial r}\right) + \frac{\partial}{\partial \theta}\left(\frac{D}{r}\frac{\partial c}{\partial \theta}\right) + \frac{\partial}{\partial z}\left(rD\frac{\partial c}{\partial z}\right)\right\} \quad (5)$$

Here, c and D are the concentration and diffusion coefficient of the drug; r denotes the radial coordinate, z the axial coordinate, and θ the angle perpendicular to the r–z-plane [14].

Also, the limited solubility of the drug within the system is taken into account: only drugs that are soluble under the given conditions are considered to be available for diffusion. The initial condition reflects the random distribution of the drug within the microparticles at $t = 0$, the boundary

conditions are based on the symmetry planes within the spherical microparticles at $r = 0$ and $z = 0$ and perfect sink conditions (which are maintained throughout the experiment). As the diffusion coefficient of the drug is not constant the respective set of partial differential equations was solved numerically, using finite differences.

Analogous to the simplified approach, the complex model was fitted to the experimentally determined in vitro drug release rates from microparticles, which were exposed to different γ-irradiation doses. Based on a quantitative relationship between the initial drug diffusion coefficient (D_0) and the applied dose the resulting drug release kinetics from microparticles exposed to 4.4 kGy were theoretically predicted and compared to experimental results. For the implementation of the mathematical model the programming language C++ was used (Borland C++ 6.0).

5. Results and discussion

5.1. Effect of γ-irradiation on the physicochemical properties of the microparticles

The effect of different γ-irradiation doses on the average molecular weight of the polymer, M_w, in drug-free and 5-FU-loaded microparticles as well as in PLGA powder is shown in Fig. 1A. The irradiation dose was varied from 0 to 28 kGy. As expected, the polymer molecular weight decreased with increasing irradiation dose due to the induced ester bond cleavage [1]. The decrease was almost linear, the average molecular weight dropped from approximately 104 to around 70 kDa. Interestingly, the values obtained with drug-free and 5-FU-loaded microparticles as well as with PLGA powder were very similar, indicating that the microparticle preparation process (with or without drug) did not significantly alter the average polymer molecular weight or the influence of the applied γ-irradiation dose.

Fig. 1B illustrates the effect of the γ-irradiation dose on the glass transition temperature of the polymer, T_g. Drug-free and 5-FU-loaded microparticles as well as PLGA powder were studied, the γ-irradiation dose was varied from 0 to 28 kGy. Importantly, the glass transition temperature was not significantly affected in any case, remaining constant at around 40 °C. It has to be pointed out that these studies were performed with dry microparticles and dry polymer powder, respectively. Recently, it was shown that the T_g is lowered to approximately 30 °C upon imbibition of water, the latter acting as a plasticizer [13]. Thus, the polymer is in the rubbery state during drug release, allowing much higher drug diffusion rates than in the glassy state.

Scanning electron microscopy pictures of non-irradiated and γ-irradiated (33 kGy), 5-FU-loaded, PLGA-based microparticles are shown in Fig. 2. Clearly, the surfaces of the microparticles were smooth, whether the devices were

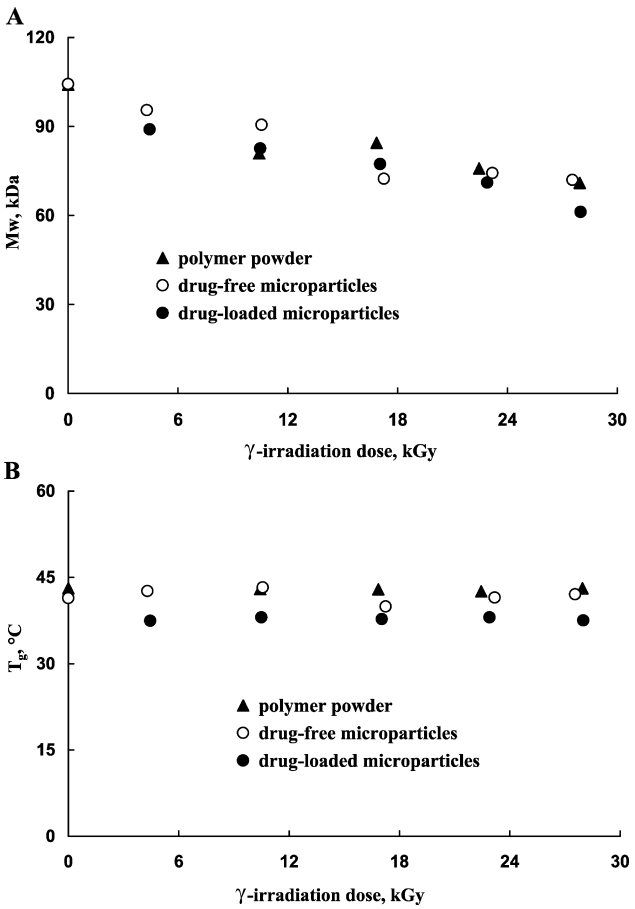

Fig. 1. Effect of the applied γ-irradiation dose (^{60}Co source) on (A) the average molecular weight (M_w); and (B) the glass transition temperature (T_g) of the bioerodible polymer PLGA (Resomer RG 506) in 5-FU-loaded and drug-free microparticles as well as on PLGA in the form of free powder.

exposed to γ-irradiation or not. Also, the inner structure of the systems was not affected by this treatment method (data not shown). 5-FU crystals were randomly distributed within the microparticles, located in cavities of different size. Table 1 shows the average diameters of drug-free and 5-FU-loaded, PLGA-based microparticles, which were exposed to different γ-irradiation doses (ranging from 4 to 33 kGy). Clearly, the microparticle size was not affected by γ-irradiation, irrespective of whether drug was incorporated or not. Furthermore, it can be seen that the 5-FU-loaded systems were significantly larger than drug-free devices (57–59 vs. 30–33 μm). This might be attributable to the different conditions during the formation of the emulsion droplets. The presence of dispersed 5-FU crystals within the organic phase probably leads to increased droplet sizes, resulting in larger microparticles upon solvent evaporation.

The effects of the applied γ-irradiation dose on the experimentally determined in vitro drug release kinetics from PLGA-based microparticles in phosphate buffer pH 7.4 are shown in Fig. 3. The release patterns of 5-FU from devices exposed to 11, 17, 23, 28 and 33 kGy are illustrated. As expected, drug release became faster with increasing

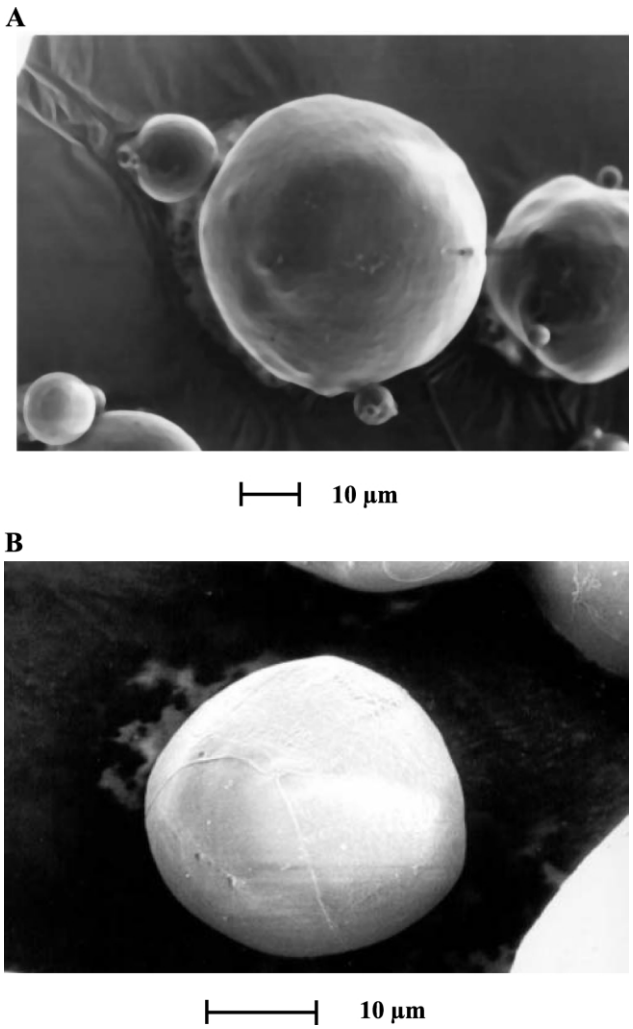

Fig. 2. SEM pictures of 5-FU-loaded, PLGA-based microparticles: (A) before; and (B) after γ-irradiation (applied dose: 33 kGy, ^{60}Co source).

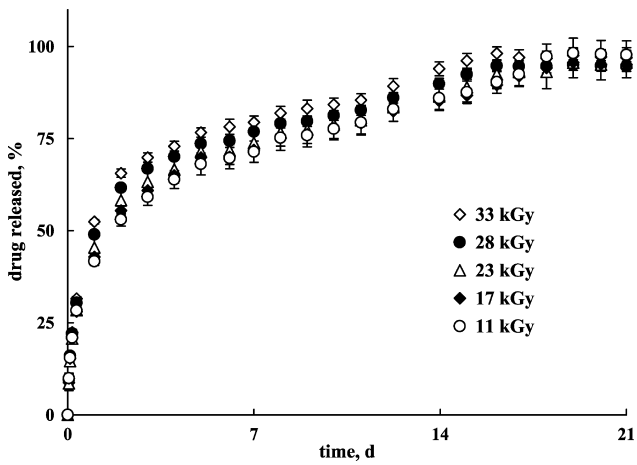

Fig. 3. Effect of the applied γ-irradiation dose (given in the figure legend, ^{60}Co source) on the experimentally measured in vitro release kinetics of 5-FU from PLGA-based microparticles in phosphate buffer pH 7.4 (reproduced from ref. [6]).

γ-irradiation dose (as each experiment was conducted in triplicate only, no statistical method was used to analyze the significance of the observed differences). This can be attributed to the irradiation induced ester bond cleavage and the resulting decrease in polymer molecular weight (Fig. 1A). The decrease in the latter has an important effect on the mobility of the macromolecules and, thus, on the free volume available for water and drug diffusion [22]: with decreasing chain length, the degree of macromolecule entanglement decreases. According to the free volume theory of diffusion, the probability for a water or drug molecule to jump from one cavity into another consequently increases. Hence, the respective diffusion coefficients and drug release rates increase.

The observed in vitro drug release kinetics from the microparticles were all biphasic: an initial rapid drug release phase ('burst') was followed by a constant, approximately zero order drug release phase (Fig. 3). The 'burst' can probably be attributed to purely diffusion controlled mass transport processes at early time points [14]. In contrast, the subsequent, almost zero order drug release phase results from the overlapping of several effects: (i) the increase in the diffusion pathways with time, which is at least partially compensated by (ii) the increase in device porosity (due to polymer degradation), and (iii) the maintenance of approximately linear drug concentration gradients over prolonged periods of time within the microparticles (due to the high 'initial drug content/drug solubility' ratio) [14]. Interestingly, at lower γ-irradiation doses the beginning of a second rapid drug release phase (at late time points) was observed. This effect can probably be attributed to the breakdown of the polymer network/disintegration of the microparticles due to hydrolytic degradation [14]. If drug is still present within the devices at this time point, the diffusion pathways significantly decrease, the surface area increases and—due to the increased porosities—the diffusivities increase, resulting in increased release rates. However, when higher γ-irradiation doses were applied, the polymer molecular weight at time $t = 0$ (before exposure to the release medium) was significantly decreased (Fig. 1A), leading to sufficiently high drug diffusion coefficients to provide complete drug exhaust before the breakdown of the polymeric structure/microparticle disintegration.

5.2. Simplified mathematical model

Rather good agreement between the fittings of the simplified mathematical model and the experimentally determined in vitro drug release from 5-FU-loaded, PLGA-based microparticles irradiated with 11, 17, 23, 28 or 33 kGy was obtained recently [6]. However, drug release was systematically underestimated at early time points, and overestimated at late time points. This can be explained by the simplifications that are made in this mathematical approach. For example, linear steady state drug

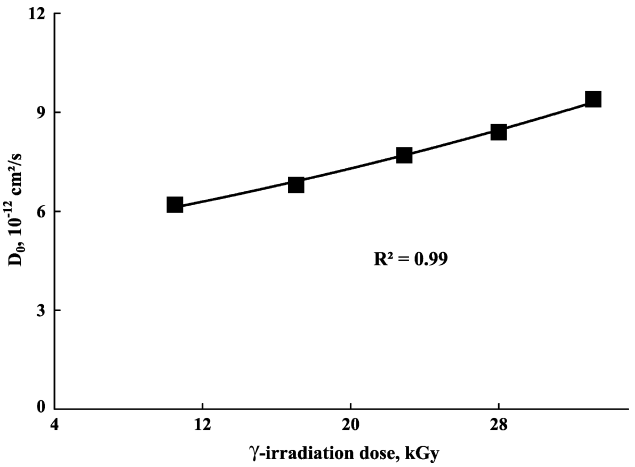

Fig. 4. Dependence of the initial diffusion coefficient of 5-FU within bioerodible, PLGA-based microparticles (D_0) on the applied γ-irradiation dose (^{60}Co source) calculated using the simplified mathematical model.

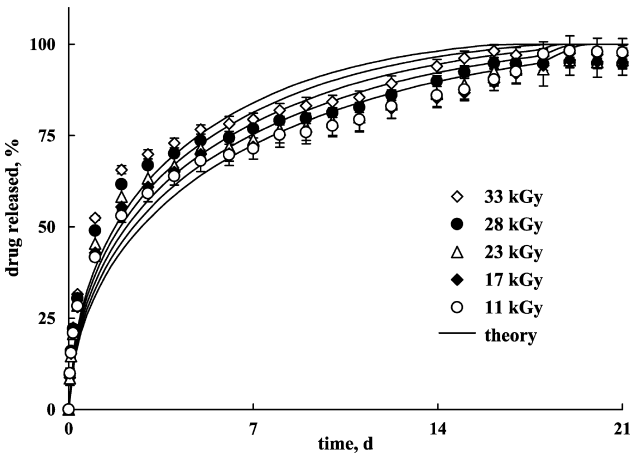

Fig. 5. Fittings of the complex mathematical model to the experimentally determined in vitro release kinetics of 5-FU from PLGA-based microparticles in phosphate buffer pH 7.4 (applied γ-irradiation dose given in the figure legend) (symbols: experimental results; curves: theoretical values).

concentration gradients are assumed and the effect of the degradation of the polymer is considered in a simplistic way only. Based on these fittings, the diffusion coefficients of the drug within the PLGA-based microparticles at time $t = 0$ (before exposure to the release medium, D_0), could be determined for each γ-irradiation dose (Fig. 4). The values ranged from 6.2 to 9.4×10^{-12} cm^2/s (for 11–33 kGy). Interestingly, an exponential equation was found to best describe the relationship between the initial drug diffusivity (D_0) and the applied γ-irradiation dose (*dose*, in kGy) (coefficient of determination, $R^2 = 0.99$):

$$D_0 = 5.04exp(0.02dose)10^{-12}\ cm^2/s \quad (6)$$

Importantly, this equation can be used to calculate the initial drug diffusion coefficient (D_0) for arbitrary γ-irradiation doses.

5.3. Comprehensive mathematical model

Fig. 5 shows the fittings of the more complex mathematical model to the experimentally determined in vitro drug release kinetics from 5-FU-loaded, PLGA-based microparticles irradiated with 11, 17, 23, 28 or 33 kGy. Clearly, the agreement between the theoretical and experimental values was better than in the case of the simplified model [6]. This can be attributed to the more realistic description of polymer erosion and drug diffusion (with time-, position-, and direction-dependent diffusion coefficients). Importantly, also the beginning of the second rapid drug release phase at late time points observed with microparticles exposed to low γ-irradiation doses could be described with the comprehensive model.

Based on the fittings shown in Fig. 5, the initial drug diffusion coefficients (at $t = 0$) within the PLGA-based microparticles were determined (Fig. 6). The obtained values range from 13 to 21×10^{-12} cm^2/s (for 11–33 kGy), being significantly higher than those determined with the simplified model ($6.2–9.4 \times 10^{-12}$ cm^2/s). This can be explained by the different time-dependencies of the drug diffusivity, which are considered in the two models. As the agreement between theoretical and experimental values at early time points was better in the case of the complex model compared to the simplified approach, it can be assumed that the diffusion coefficients obtained with the comprehensive model are physically more realistic than the diffusion coefficients obtained with the simplified model. In contrast to the simplified approach, the complex one considers the randomness of polymer erosion, directly relates the drug diffusivities and system porosities and takes the heterogeneity of the microparticles into account. Importantly, also with the complex model a quantitative relationship between the initial diffusion coefficient of the drug within the microparticles (D_0) and the applied

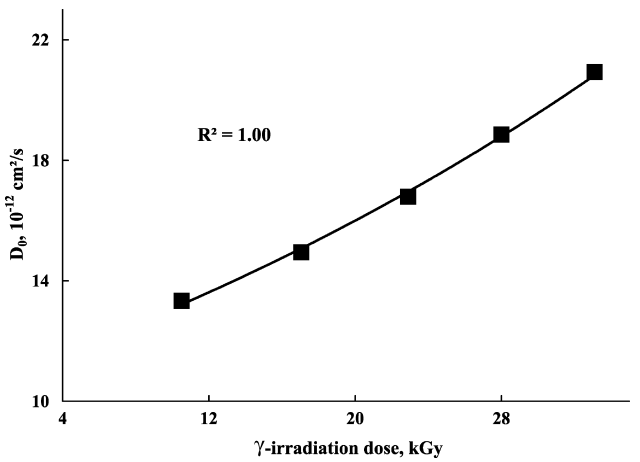

Fig. 6. Dependence of the initial diffusion coefficient of 5-FU within bioerodible, PLGA-based microparticles (D_0) on the applied γ-irradiation dose (^{60}Co source) calculated using the complex mathematical model.

γ-irradiation dose (*dose*, in kGy) could be established ($R^2 = 1.00$):

$$D_0 = 10.7exp(0.02dose)10^{-12}\ cm^2/s \quad (7)$$

As in the case of the simplified model, the diffusivity depends in an exponential manner on the irradiation dose. Analogous to Eq. (6), Eq. (7) allows the calculation of the resulting initial drug diffusivity for arbitrary irradiation doses.

5.4. Theoretical predictions and experimental evaluation

Using Eqs. (6 and 7) the diffusion coefficient of 5-FU in PLGA-based microparticles can be calculated as a function of the applied γ-irradiation dose. For a γ-irradiation dose of 4.4 kGy, the obtained D_0 values are equal to 5.5 and 11.7×10^{-12} cm^2/s (simplified and complex model, respectively). Based on these values, the two mathematical approaches were used to predict the resulting in vitro drug release kinetics from PLGA-based microparticles irradiated with 4.4 kGy in phosphate buffer pH 7.4. The theoretically predicted drug release patterns are shown in Fig. 7 (dotted curve: simplified model; solid curve: comprehensive model). In contrast to the simplified model, the complex approach predicts the beginning of a second rapid drug release phase (after approx. 18 days). These theoretical predictions were then compared to the experimentally measured in vitro drug release rate from 5-FU-loaded, PLGA-based microparticles exposed to 4.4 kGy [6]. As can be seen in Fig. 7, the calculations could be confirmed by the experimental results. As expected, the theoretical predictions calculated with the comprehensive mathematical model were more accurate than those calculated with the simplified approach.

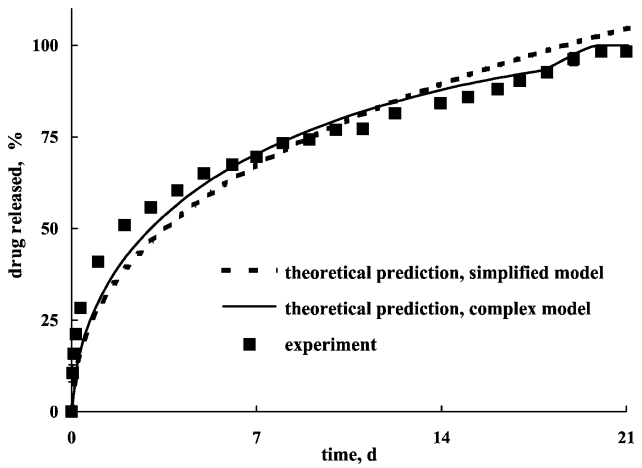

Fig. 7. Theoretical prediction and experimental evaluation of the in vitro drug release kinetics of 5-FU from PLGA-based microparticles exposed to 4.4 kGy (^{60}Co source) in phosphate buffer pH 7.4 (dotted curve: simplified mathematical model; solid curve: complex mathematical model; symbols: experimental results).

6. Conclusions

The effects of γ-irradiation on the physicochemical properties of drug-loaded, PLGA-based microparticles, such as the polymer molecular weight, glass transition temperature, morphology, particle size and in vitro drug release kinetics were studied. Based on these experimental results, two mathematical models—a simplified and a more comprehensive approach—considering polymer degradation and drug diffusion were used to describe the observed in vitro drug release behavior. Importantly, quantitative relationships between the applied γ-irradiation dose and the resulting initial drug diffusion coefficient within the systems could be established. Using these relationships it was possible to predict the resulting drug release kinetics for arbitrary irradiation doses. Importantly, the theoretical predictions could be confirmed by experimental results.

Acknowledgements

This work was supported by the European Commission (Marie Curie Individual Fellowship, Contract No. HPMF-CT-1999-00033) (Research and Technological Development Project BCDDS, Contract No. QLK3-CT-2001-02226). Furthermore, the authors are grateful to R. Filmon (Service Commun de Microscopie Electronique, Faculté de Médecine, Université d'Angers, Angers, France) for the SEM pictures, and to J. Haffner-Colleau (INSERM ERIT M 0104), V. Laffineur and O. Thomas (Ethypharm) for their valuable assistance.

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