Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutics and Biopharmaceutics 57 (2004) 189–197
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Development of novel 5-FU-loaded poly(methylidene malonate 2.1.2)-based microspheres for the treatment of brain cancers

Elvire Fournier[a,b,1], Catherine Passirani[b], Nathalie Colin[a], Pascal Breton[a], Serge Sagodira[a], Jean-Pierre Benoit[b,*]

[a]*Virsol, Paris, France*
[b]*Inserm ERIT-M 0104 "Ingénierie de la Vectorisation Particulaire", Angers, France*

Received 10 January 2003; accepted in revised form 11 August 2003

Abstract

In order to treat malignant brain tumors by local delivery of antineoplastic agents, the feasibility of 5-fluorouracil (5-FU)-sustained release biodegradable microspheres with a novel material, poly(methylidene malonate 2.1.2), was investigated using an emulsion/extraction method. This polymer was expected to present a slow degradation rate, thus leading to a long term local delivery system. Microparticles were successfully obtained and characterized in terms of drug loading, size, morphology and release profile. The size of the particles was between 40 and 50 μm, which was compatible with a stereotactic injection through a needle. Sufficient drug loadings were obtained (i.e. compatible with the preparation of therapeutic 5-FU doses in a minimal volume of injection), and perfectly spherical microspheres were observed. The respective influences of the polymer molecular weight, the polymer concentration, and the emulsion time on the release profiles were studied using a 2^3 factorial design. In the same objective, the solvent extraction time was extended while keeping all the previous parameters fixed at their optimal values. The in vitro study of these different parameters allowed a reduction of the initial burst release, with a percentage of 5-FU released after 24 h that was lowered from 90 to 65%, and the achievement of a long term drug delivery system, since the release was still ongoing after 43 days. Moreover, the microparticles could be gamma-sterilized (25 kGy) without modification of the release kinetics. Thus, the requested specifications to perform animal experiments were attained.

Keywords: Poly(methylidene malonate 2.1.2); Microsphere; 5-Fluorouracil; Long term delivery; Experimental design

1. Introduction

Malignant gliomas are aggressive human brain tumors, often affecting the young. Among them, glioblastomas, also called high-grade gliomas, represent approximately 85% of malignant gliomas. They have a low incidence of 5–8/100,000 inhabitants/year [1], but the median survival does not exceed 1 year, even if treated [2–4]. Current treatment consists of surgical resection of the tumor, when its localization allows it, followed by radiotherapy and/or chemotherapy. No clinical trial has yet demonstrated a significant advantage of neo-chemotherapy delivered before radiotherapy [1]. Moreover, those tumors have indistinct margins and cannot be completely removed. Thus, they tend to recur within centimeters of their original location. Usually, drug diffusion in brain tissue is limited to the vicinity of the implantation site [5,6]. Therefore, glioblastoma, which is notable for its unifocal character and for its low metastatic potential, tends itself to local antineoplastic treatment. Indeed, the main reason for treatment failure is very often an evolution of the tumor in, or very close to, the initial site of the primitive tumor [7]. The therapeutic arsenal is also limited by the presence of the blood–brain barrier.

In the last few years, extensive research has focused on finding new effective drugs and/or new ways of drug targeting to improve drug delivery to the central nervous system (CNS). One approach is enhancement of the local concentration of chemotherapeutic agents, by implantation of biodegradable polymeric devices in the tumor or in the resection cavity. The idea is to assure a sustained release of the drug to avoid recurrences within several centimeters of

* Corresponding author. Inserm ERIT-M 0104 "Ingénierie de la Vectorisation Particulaire", 10 rue André Bocquel, 49 100 Angers, France.
[1] Present address: Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University of Montreal, Montreal (QC), Canada.

0939-6411/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0939-6411(03)00146-2

the initial location. Even though microencapsulated drug diffusion distances are short, glioma features should make local delivery a good approach, given that microspheres seem particularly suitable for direct implantation in a tumor because they spontaneously remain in situ in tumorized areas [8]. Moreover, a multi-point administration can easily be performed during surgery or by stereotaxy.

Brem et al. (1991) incorporated 1,3-bis-(-2chloroethyl)-1-nitrosourea (BCNU) in wafers constituted of poly[bis(p-carboxyphenoxy)]propane-sebacic acid copolymer (PCPP-SA) and first carried out a phase I–II dose escalation study showing safety and promising efficacy on recurrent gliomas [9]. Thereafter, they initiated a prospective, randomized, placebo controlled trial of safety and efficacy of intraoperative controlled delivery of chemotherapy [10]. The wafers were left on the walls of the resection cavity. In this study, like in many following studies, patient survival was significantly improved. Nevertheless, tumor recurrences could not be prevented and were only delayed. Moreover, the discoid wafers can only be used after tumor resection, thus implicating an operable tumor [11]. In parallel, Menei et al. (1994) developed a new concept of drug targeting into the CNS by stereotactic implantation of biodegradable microspheres [12]. Because of their size, these microparticles can easily be implanted by stereotaxy in functional areas of the brain. Compared to large implants, microparticles do not need open surgery. A phase I pilot study of the local and sustained delivery of 5-fluorouracil (5-FU) was carried out in eight patients with newly diagnosed glioma [13]. Microspheres were implanted after a complete macroscopic surgical resection of the tumor. Patient median survival was doubled and one patient is still in disease remission today. Based on these results, a multi-institutional phase IIb clinical trial is presently ongoing [14].

In order to improve this strategy, we focused on a new material, poly(methylidene malonate 2.1.2) (PMM 2.1.2), described by Bru-Magniez et al. (1990) and Lescure et al. (1991) [15,16]. The main advantage expected from this novel polymer is a long degradation time, due to its chemical structure (ester bonds in the side chains only), which could be advantageous in the treatment of long evolving pathologies, avoiding repetition of stereotactic injections. Indeed, in a parallel biocompatibility study of PMM 2.1.2, microspheres were injected into Sprague–Dawley female rats, and were still perfectly intact at 3 months (data not shown). Therefore, we could expect a very interesting system, which could firstly degrade much more slowly than poly(lactide-co-glycolide) polymer (PLGA) [17], thus assuring a much more prolonged local release of a drug in a specific area of the brain tissue, and secondly extend the panel of biocompatible synthetic polymers available in the literature [18].

The aim of this work was to develop 5-FU-loaded PMM 2.1.2-based microspheres with an appropriate size for stereotactic injection. 5-FU was chosen because (1) it weakly passes the blood–brain barrier, (2) after intravenous injection, its half-life is less than 20 min, (3) it is mainly active on highly dividing cells, which are not encountered in the CNS, and is therefore rarely neurotoxic, and (4) it is a potent radiosensitizer.

Size, morphology, drug loading of the microspheres and 5-FU localization and release from the particles were studied. Thereafter, the influence of the polymer molecular weight and concentration and the emulsion time were studied in order to improve the 5-FU release profiles. Concomitantly, the effects of an increased solvent extraction time were evaluated.

2. Materials and methods

2.1. Polymer synthesis and molecular weight determination

The monomer 1-ethoxycarbonyl-1-ethoxycarbonyl-methylenoxycarbonyl ethane (methylidene malonate 2.1.2 or MM 2.1.2) was prepared according to Bru-Magniez et al. (1990) and Lescure et al. (1991, 1994) [15,16,19]. During storage, it was maintained under a sulfur dioxide (SO_2) atmosphere at -18 °C to prevent spontaneous polymerization.

Before polymerization of the monomer, SO_2 was removed by low vacuum during 3 h. NaOH 0.1 N (Sigma, St Quentin Fallavier, France) was used as the initiator. SO_2-free monomer (4.5 g before SO_2 removal) was dissolved in acetone (75 ml, Prolabo, Strasbourg, France) and NaOH was then added under magnetic stirring. Although polymerization occurred after 5 min, stirring was maintained during 15 min to assure a complete reaction. Acetone was evaporated under vacuum (100 rev./min, 43 °C). Finally, a large volume of deionized water (800 ml) was added to collect the polymer, which was filtered and lyophilized. According to the amounts of NaOH added to initiate the polymerization, different molecular weights could be obtained [20].

The molecular weight of the synthesized PMM 2.1.2 was determined by size exclusion chromatography (SEC) in tetrahydrofuran 84.6%/methanol 14.6%/acetic acid 0.8% as the elution phase (flow rate of 1 ml/min). Two columns in series and a pre-column were used (GPC column Plgel®, 5 μm, 10^4 Å, 300 × 7.5 mm, Polymer Laboratories, Marseille, France; GPC column Styragel® HR1, 300 × 7.8 mm; pre-column Shodex® KF-G type, Waters, France). Detection was performed using a refractometer (RID-10A Shimadzu, Touzard et Matignon, Courtaboeuf, France) and molecular weights were calculated with GPC software (PL-caliber, Polymer Laboratories, Marseille, France). Results were expressed as number, weight and peak average molecular weights $\overline{Mn}$, $\overline{Mw}$ and $\overline{Mp}$, respectively (polystyrene standards).

2.2. Microsphere preparation

The microspheres were prepared using an emulsion/extraction method. The emulsion and the extraction steps took place in a reactor in which temperature was under control. This technique is based on the formation of an oil-in-water emulsion obtained under controlled stirring. 5-FU crystals (Roche Laboratories, Neuilly sur Seine, France) were ground [21] and 300 mg were suspended in 6 ml of methylene chloride (Prolabo, Strasbourg, France) using an UltraTurrax® homogenizer for 2 min (9500 rev./min). The polymer (300 or 600 mg) was then dissolved in the organic solvent under magnetic stirring, which contributed to maintaining the 5-FU in suspension. The suspension was driven to a temperature lower than 5 °C. An emulsion was obtained by pouring the organic phase into a polyvinyl alcohol (PVA, 88% hydrolyzed, Rhodoviol® 4/125, Prolabo, Strasbourg, France) aqueous phase (200 ml, 2% w/v, 2 °C) under mechanical stirring (600 rev./min, 3 min). Deionized water (1300 ml, 4 °C) was then added to the emulsion, allowing the formation of microspheres, which resulted from the solvent extraction (500 rev./min, 2 min) [22].

Thereafter, the particles were collected by filtration under nitrogen pressure (filter Durapore HVLP 0.45 μm, Millipore, St Quentin en Yvelines, France), washed with deionized water, resuspended in a minimum of deionized water, frozen in liquid nitrogen and freeze-dried (freeze-drier RP2V, Sérail, Argenteuil, France).

2.3. Microsphere characterization

2.3.1. Determination of 5-FU content

5-FU-loaded microspheres (10 mg) were dissolved in 50 ml dimethylsulfoxide (Prolabo, Strasbourg, France), and the optical density was read at 266 nm by spectrophotometry (Uvikon 940, Kontron, St Quentin en Yvelines, France). The assays were performed in triplicate and expressed in terms of weight of 5-FU per weight of microspheres, thus determining the actual encapsulation ratio (AER) defined by the following expression:

$$\text{AER} = \text{measured drug weight/microsphere weight}$$

The theoretical encapsulation ratios (TER) were also calculated in order to determine the encapsulation efficiency:

$$\text{TER} = \text{drug weight/(drug weight} + \text{polymer weight)}$$

$$\text{Encapsulation efficiency} = \text{AER/TER}$$

2.3.2. Size distribution analysis

The particle sizes were determined by using a Coulter® counter (Multisizer, Coultronics, Margency, France). Microparticles (10–20 mg) were suspended in deionized water (5 ml) and assayed after dilution in Isoton® II (Coultronics, France).

2.3.3. Microscopy studies

Microspheres were examined with an Axioscope® 2 optical microscope (Zeiss, Le Pecq, France). Different samples were viewed by scanning electron microscopy (SEM) and fluorine cartographies were realized (Jeol JSM 6301F, Jeol, Croissy sur Seine, France) on microspheres which were broken with an adhesive before metallization [23]: freeze-dried microspheres were mounted onto metal stubs using double-sided adhesive tape, vacuum-coated with a carbon film (MED, Bal-Tec, Balzers, Liechtenstein) and directly analyzed under SEM. To characterize the internal morphology and 5-FU localization, the adhesive tape with stuck particles was firstly folded on itself and secondly roughly unfolded to fracture the microspheres. The coating was carried out as previously described.

2.3.4. In vitro release studies

Kinetics studies were performed in a USP dissolution apparatus shaken by a paddle at 100 rev./min (Sotax AT7, Basel, Switzerland) at 37 °C in PBS (pH 7.4). A dialysis bag containing 30 mg microparticles was filled with PBS and placed in 500 ml of this buffer. 5-FU released from microspheres was assayed spectrophotometrically at 266 nm. Each batch of microparticles was examined in triplicate.

2.4. Optimization of 5-FU in vitro release kinetics

A 2^3 full factorial design was carried out to study the influence of three parameters (polymer molecular weight, polymer concentration, emulsion time) on the kinetics of in vitro release and on the extent of drug loading. For each parameter, two levels were fixed, one inferior level (−1) and one superior level (+1) (Table 1). The experiments were carried out in a random order to minimize the effect of systematic errors. The experimental results were analyzed using Nemrod® software [24].

Finally, the influence of the solvent extraction time was also explored, between 2 and 10 min, with 2 min increments. All the experiments were done in triplicate.

Table 1
Studied parameters

Coded variable	Variable	Level	
		−1	+1
X_1	Polymer molecular weight	17,673 g/mol	24,135 g/mol
X_2	Polymer concentration	5%	10%
X_3	Emulsion time	3 min	5 min

2.5. Microsphere sterilization

Several batches of microspheres were sterilized by γ-irradiation (Ionisos, Dagneux, France) at 25 kGy, and examined in terms of PMM 2.1.2 molecular weights, drug loading and kinetics.

3. Results

3.1. The preliminary batches of microspheres

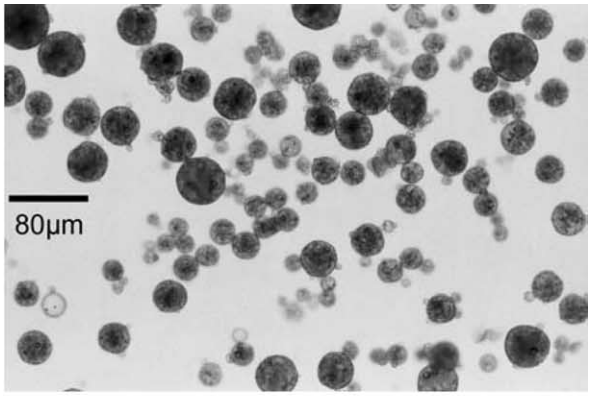
Optical and scanning electron micrographs both showed that microspheres were perfectly spherical. Fig. 1 reveals 5-FU crystals located both inside and at the surface of the particles. Drug loadings of the microspheres were between 10 and 25% and the encapsulation yields between 20 and 75%. The average microsphere diameter was always between 40 and 50 μm. Before optimization, more than 90% of 5-FU was released within 24 h in vitro (Fig. 2).

3.2. The critical factors

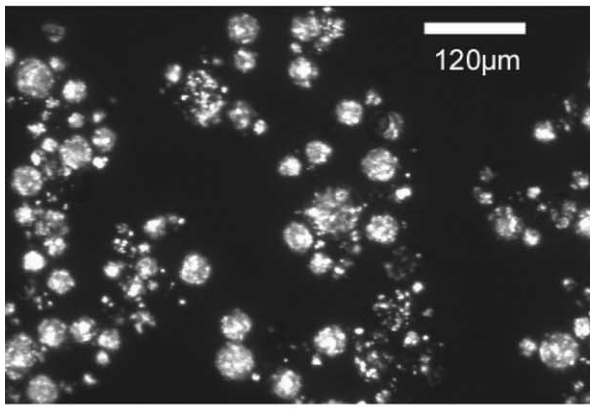
Experiments defined by the full factorial design and corresponding results are presented in Table 2. The encapsulation ratios measured were between 10.4 and 19.1%. The best kinetics were around 65% of 5-FU released after 24 h (Fig. 2) and the release was still ongoing after 43 days. At day 43, microparticles were not significantly degraded when observed by optical microscopy (Fig. 3), compared to native microspheres. The eight distinct experiments of the full factorial design allowed us to calculate main effects of each parameter on the studied responses but also the interactions between them. For the encapsulation ratio no significant effect of any parameter was observed. The effects calculated for 5-FU released (Y_2) are shown graphically in Fig. 4a. The higher the effect of a factor in absolute value, the more significant the effect it had on 5-FU release. The estimation of the experimental variance ($S^2_{y2} = 7.33$) allowed us to calculate the significance of individual coefficients by means of Student's t variable with 2 degrees of freedom ($\alpha = 0.05$) [25]. A significant second order interaction between the three factors was observed so they had to be considered together. Three dimensional representation of the eight experiments made it possible to draw some conclusions (Fig. 4b). The numbers at the corner of the design box indicate the results of experiments.

Given that a good kinetics is one with the lower possible values at a given corner of the box, from this diagram, it could be concluded that to obtain the best kinetics:

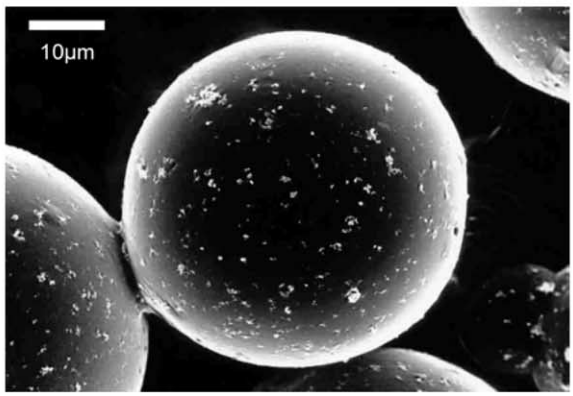
- PMM 2.1.2 molecular weight must be close to its high level, that is 24,135;
- the polymer concentration can be high or low, but for a high concentration (10%) the emulsion time must be at its low level (3 min);
- the emulsion time can be high or low, but for a high emulsion time (5 min) the polymer concentration must be at its low level (5%).



a



b



c

Fig. 1. Optical micrographs in normal light (a) or polarized light (b) and scanning electron micrograph of 5-FU-loaded PMM 2.1.2-based microspheres (c).

Drug loadings were higher when the emulsion time was at its low level.

The morphology of the microsphere interior is represented in Fig. 5 which shows that 5-FU is mostly located in the core of the spheres. This could be confirmed by a fluorine cartography: Fig. 5b is the fluorine map of the same microsphere as in Fig. 5a. Each point corresponds to fluorine

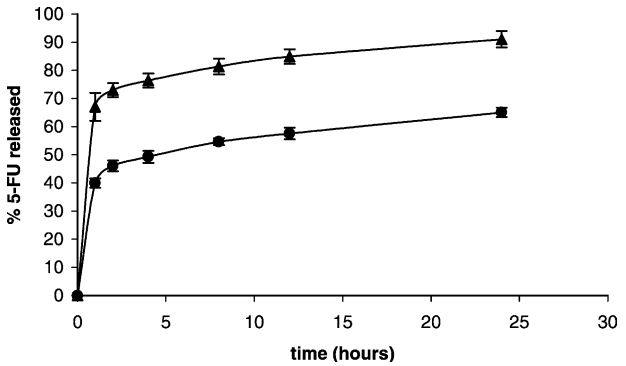


Fig. 2. In vitro release profile of 5-FU from microparticles before (▲) and after (●) the factorial design.

presence and it appears clearly that fluorine is mostly located in the center of the sphere.

3.3. The solvent extraction time

This parameter has been explored while all the other parameters were fixed at the values giving the best kinetics. The solvent extraction time varied from 2 to 10 min. The drug loading did not vary significantly from 2 to 8 min, but decreased below 15% for a 10 min extraction (Fig. 6a). Fig. 6b represents the influence of the solvent extraction time on the burst effect. In a first step, the percentage of 5-FU released within the early stage increased when the solvent extraction time was increased. It stayed high from 4 to 8 min, and decreased again for a 10 min extraction.

3.4. γ-Irradiation

The PMM 2.1.2 molecular weights, the drug loading and the percentage of 5-FU released after 24 h of sterilized and not sterilized particles are shown in Table 3. A 15.4% and 16.1% loss of number and weight average molecular weight were observed, respectively. The drug loading and the percentage of 5-FU released after 24 h were unchanged.

Table 2
Factorial design and experimental results

PMM 2.1.2 $\overline{Mw}$ (g/mol)	PMM 2.1.2 concentration (%)	Emulsion time (min)	Drug loading (%)	% 5-FU released ($t = 24$ h) (%)
17673	5	3	18.5	89.3
24135	**5**	**3**	**17.3**	**68.7**
24135	**5**	**3**	**15.1**	**63.7**
24135	**5**	**3**	**17.9**	**64.4**
17673	10	3	14.8	79.3
24135	10	3	15.4	64.8
17673	5	5	19.1	92.9
24135	5	5	13.1	65.1
17673	10	5	16.4	71.7
24135	10	5	10.4	91.1

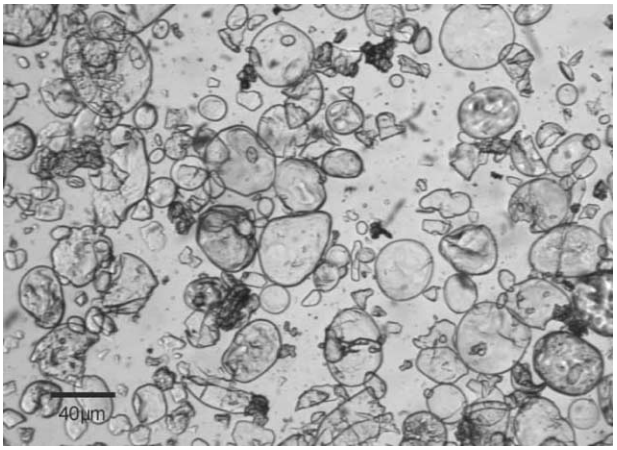


Fig. 3. Optical micrograph of 5-FU-loaded PMM 2.1.2-based microspheres after 43 days in vitro (PBS, pH 7.4, 37 °C).

4. Discussion

4.1. Influence of the polymer molecular weight, the polymer concentration and the emulsion time on the 5-FU release profile

The molecular weight ($\overline{Mw}$) of a polymer classically affects the release kinetics by influencing the degradation rate of the polymer [26] or the organic phase viscosity,

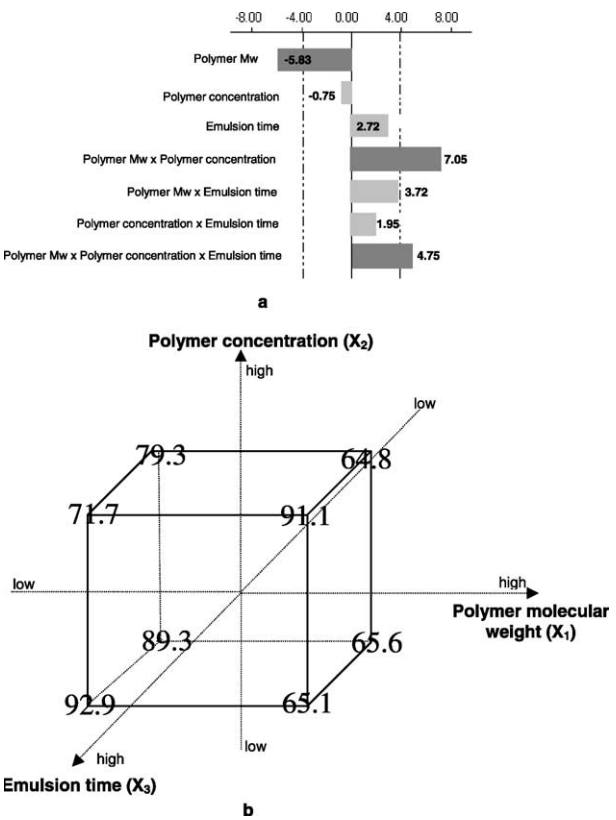


Fig. 4. Graphical analysis of the parameter effects on 5-FU release (a). Interaction diagram for PMM 2.1.2 molecular weight (X_1), PMM 2.1.2 concentration (X_2) and emulsion time (X_3) (b) (each corner corresponds to the percentage of 5-FU released after 24 h).

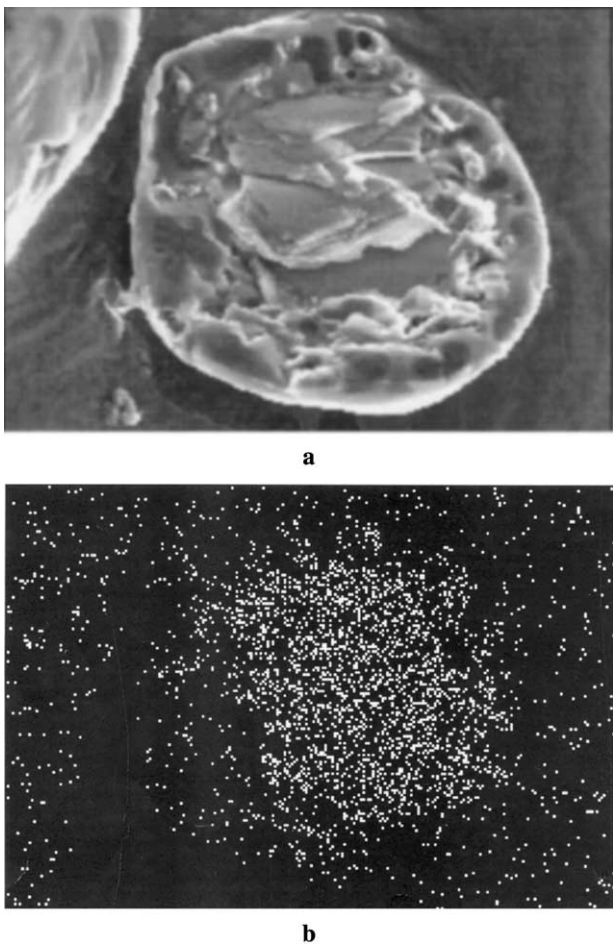


Fig. 5. Scanning electron micrograph of a 5-FU-loaded PMM 2.1.2-based microsphere showing (a) interior and (b) fluorine cartography.

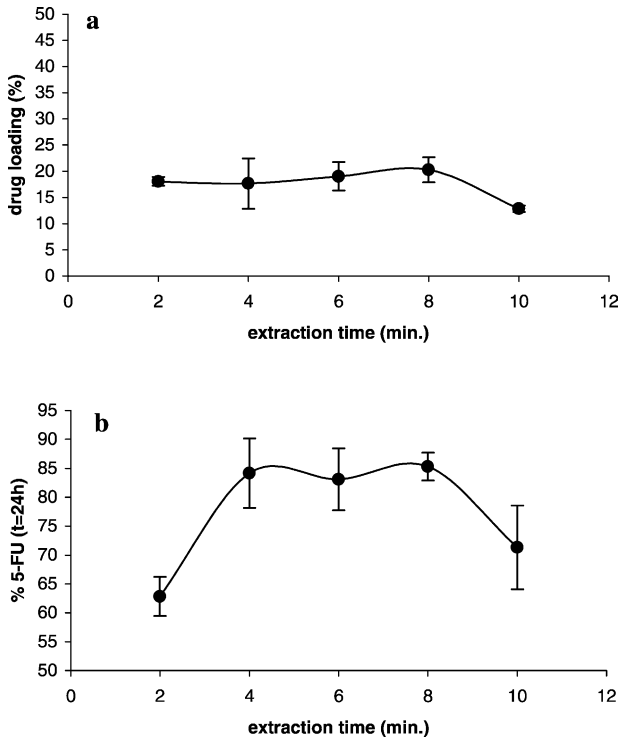


Fig. 6. Influence of the extraction time on the drug loading (a) and on the 5-FU release rate (b).

which can act upon the crystal distribution in the polymer matrix [27]. Indeed, increasing the organic phase viscosity prevents the diffusion of the crystals towards the surface of the particles, which is a consequence of the centrifugation force due to the stirring. And the less crystals are present at the periphery, the less the initial burst is pronounced. In this study, a triple interaction between the parameters was observed: increasing the molecular weight resulted in lowering the release of the drug (except for a high polymer concentration and a high emulsion time). Microparticles were not significantly degraded in vitro at day 43, thus showing a minor contribution of the polymer degradation rate to the release of 5-FU, and suggesting a main release by diffusion through the polymer matrix, as previously observed [28].

Usually, increasing the polymer concentration leads to a more dense polymer matrix attenuating the burst effect because the migration of the encapsulated drug is restricted [29–32]. In this study, satisfactory kinetics could be obtained for both high or low levels of polymer concentration, depending on the other parameters (triple interaction). Indeed, both high and low concentrations can allow a reduction of the burst effect: Jalil et al. (1990) explained that for high concentrations, the precipitation of a polymer is faster and can lead to surface defects responsible for a burst effect [33]. Thus, a low concentration may be preferable. On the contrary, Hu et al. (2000) observed that for high concentrations, strong interactions between the encapsulated protein and the polymer can achieve a better sustained release [34]. Therefore, an alternative to slow down the release of 5-FU could be the co-encapsulation of 5-FU with a protein of therapeutic interest in the treatment of brain cancers. Hussain et al. (2002) recently co-encapsulated an antisense oligonucleotide (AON) with 5-FU and showed that 5-FU release was decreased by the presence of the AON [35].

The emulsion time can play an important role in the internal morphology of the microspheres, thus having an impact on the kinetics [30]. If the emulsion time is reduced, 5-FU crystals have less time to escape from

Table 3
Characteristics of microspheres before and after γ-irradiation (25 kGy)

Characteristic	Before γ-irradiation	After γ-irradiation
PMM 2.1.2-based microsphere molecular weight (GPC)	$\overline{Mw}$ = 31,584 g/mol; $\overline{Mn}$ = 18,814 g/mol; Polydispersity Index = 1.924	$\overline{Mw}$ = 26,497 g/mol; $\overline{Mn}$ = 15,909 g/mol; Polydispersity Index = 1.970
Drug loading (%)	18.2	18.1
% 5-FU released after 24 h	75.6 ± 0.8	68.9 ± 2.0

the microspheres during the emulsion step. The drug loading and the release are therefore improved. In this study, for high polymer $\overline{Mw}$ and concentration, extending the emulsion time resulted effectively in accelerating the release of the drug. A further explanation can be that, as previously observed, when the emulsion time increases, the size of the droplets decreases, thus reducing also the size of the particles [36]. In small particles, 5-FU crystals are uniformly dispersed in the matrix. Some crystals are located at the periphery of the microspheres and the release is therefore more rapid. Moreover, small particles have a larger transfer surface area for the drug to diffuse out from the matrix [37]. In this work, particle size was not affected by the emulsion time in the range studied (data not shown), as previously observed [38]. Therefore, for a high emulsion time, crystals were able to diffuse in the forming microspheres, even if viscous, to reach the periphery and enhance the 5-FU release. For a short emulsion time, as the sustained release persisted after 43 days, at least 35–40% of the crystals were right away encapsulated in a polymer barrier during the early stage of the encapsulation process. The polymer played a plain role of diffusion barrier for the drug during the in vitro release.

In conclusion of this first optimization step, all factors had an influence on the in vitro release profiles, and their influence was inter-related, since the factorial design showed a triple interaction. Compared with 5-FU-loaded PLGA microspheres, kinetics were faster, but longer. Nevertheless, for the treatment of glioblastoma, no optimum release profile was proven to be significantly better than another, and no advantage could be evidenced from fast or slow-releasing microspheres in vivo [8]. Moreover, in humans, the 'burst effect' may be lower.

For future in vivo experiments, microspheres must be suspended in a sterile aqueous solution of carboxymethylcellulose (CMC). Given that only small volumes can be injected intracerebrally, and that only a limited quantity of microspheres can be resuspended in CMC, drug loadings must be as high as possible, or at least compatible with the administration of therapeutic 5-FU doses. The drug loadings corresponding to the best kinetics were in agreement with this criterion.

4.2. Influence of the solvent extraction time on the drug loading and the 5-FU release profile

The influence of the solvent extraction and emulsion times are inter-related, since they represent the period in which 5-FU is partitioning between the droplets and the aqueous phase. In this work, the drug loading was only affected by a 10 min extraction time. Below 10 min, the crystals which were probably right away encapsulated in a polymer barrier during the emulsion step did not reach the aqueous phase during the extraction step and did not significantly reduce the AER.

Concerning the percentage of 5-FU released after 24 h, the influence of the solvent extraction time was more complex: a 2 min extraction allowed us to obtain the best kinetics. Then, increasing the solvent extraction time led to an increased burst effect. 5-FU crystals can have started their diffusion through the polymer matrix during the extraction step. Their diffusion was stopped by freeze-drying. Thus, crystals were located close to the surface of the microspheres during the early stage of the in vitro release experiments and induced a marked burst effect. Finally, for a 10 min extraction, the release was decreased, probably in association with a very low drug loading [29].

4.3. Influence of the γ-irradiation on the PMM 2.1.2 molecular weight, the drug loading and the 5-FU release profile

Microparticle batches were γ-sterilized without any important decrease in the number average molecular weight $\overline{Mn}$ nor in the release kinetics. This is of particular interest compared to PLGAs. Indeed, PLGAs are usually strongly affected by γ-irradiation, by a mechanism of chain scission due to cleavage of the ester linkage [39]. The degradation rate of these materials is therefore enhanced, increasing also the release of the encapsulated drug. In the PMM 2.1.2 chemical structure, ester groups only exist in the side chains. This might be the reason for the polymer resistance to γ-irradiation, affecting, if at all, the side chains without backbone scission.

5. Conclusion

We succeeded in preparing 5-FU-loaded microspheres with a novel promising material, PMM 2.1.2, using an emulsion/extraction method. The particles had an appropriate size for a stereotactic injection through a needle. The drug loadings were compatible with the preparation of therapeutic 5-FU doses in adequate volumes of injection and the morphology of the particles observed by optical or scanning electron microscopy revealed microspheres perfectly regular and spherical. A 2^3 full factorial design was carried out to optimize the release kinetics and showed a combined influence of the polymer concentration, its molecular weight and the emulsion time on the 5-FU release profile. After optimization, the percentage of 5-FU released after 24 h could finally be lowered to 65% and was not increased after γ-irradiation. Microparticles were not significantly degraded in vitro after 43 days and the release was still on-going.

Thus, PMM 2.1.2 was shown to be suitable for the formulation of an efficient drug delivery system, which may be capable of delivering a drug over long periods as demonstrated recently in F98 glioma-bearing rats [40].

Acknowledgements

The authors are very grateful to Dr M.C. Venier-Julienne (Inserm ERIT-M 0104, Angers, France) for her help concerning the experimental design methodology, and Dr R. Filmon and M. Lesourd (Service Commun de Microscopie Électronique, Université d'Angers, France) for SEM analysis.

References

[1] A.A. Brandes, D. Lacombe, C. Vecht, Future trends in the treatment of brain tumours, Eur. J. Cancer 37 (2001) 2297–2301.
[2] H.A. Fine, K.B. Dear, J.S. Loeffler, P.M. Black, G.P. Canellos, Meta-analysis of radiation therapy with and without adjuvant chemotherapy for malignant gliomas in adults, Cancer 71 (1993) 2585–2597.
[3] P.L. Kornblith, M. Walker, Chemotherapy for malignant gliomas, J. Neurosurg. 68 (1988) 1–17.
[4] S.B. Green, D.P. Byar, M.D. Walker, D.A. Pistenmaa, E. Alexander, U. Batzdorf, W.H. Brooks, W.E. Hunt, J. Mealey, J.L. Odom, P. Paoletti, J. Ransohoff, J.T. Robertson, R.G. Selker, W.R. Shapiro, K.R. Smith, C.B. Wilson, T.A. Strike, Comparisons of carmustine, procarbazine, and high-dose methylprednisolone as additions to surgery and radiotherapy for the treatment of malignant glioma, Cancer Treat. Rep. 67 (1983) 121–132.
[5] S.E. Boehnke, D. Rasmusson, Time course and effective spread of lidocaine and tetrodotoxin delivered via microdialysis: an electrophysiological study in cerebral cortex, J. Neurosci. Methods 105 (2001) 133–141.
[6] U. Roelcke, O. Hausmann, A. Merlo, J. Missimer, R.P. Maguire, P. Freitag, E.W. Radu, R. Weinreich, O. Gratzl, K.L. Leenders, PET imaging drug distribution after intratumoral injection: the case for 124 I-iododeoxyuridine in malignant gliomas, J. Nucl. Med. 43 (2002) 1444–1451.
[7] R. de Crevoisier, J. Pierga, R. Dendale, L. Feuvret, G. Noel, J. Simon, J. Mazeron, Radiation therapy in the treatment of glioblastoma, Cancer/Radiotherapie 1 (1997) 194–207.
[8] V.G. Roullin, J.R. Deverre, L. Lemaire, F. Hindre, M.C. Venier-Julienne, R. Vienet, J.P. Benoit, Anti-cancer drug diffusion within rat brain tissue: an experimental study using [3H](6)-5-fluorouracil-loaded PLGA microspheres, Eur. J. Pharm. Biopharm. 53 (2003) 293–299.
[9] H. Brem, M.S. Mahaley, N.A. Vick, K.L. Black, S.C. Schold, P.C. Burger, A.H. Friedman, I.S. Ciric, T.W. Eller, J.W. Cozzens, J.N. Kenealy, Interstitial chemotherapy with drug polymer implants for the treatment of recurrent gliomas, J. Neurosurg. 74 (1991) 441–446.
[10] H. Brem, S. Piantadosi, P.C. Burger, M. Walker, R. Selker, N.A. Vick, K. Black, M. Sisti, S. Brem, G. Mohr, P. Muller, R. Morawetz, S.C. Schold, Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas, Lancet 345 (1995) 1008–1012.
[11] H.H. Engelhard, The role of interstitial BCNU chemotherapy in the treatment of malignant glioma, Surg. Neurol. 53 (2000) 458–464.
[12] P. Menei, J.P. Benoit, M. Boisdron-Celle, D. Fournier, P. Mercier, G. Guy, Drug targeting into the central nervous system by stereotactic implantation of biodegradable microspheres, Neurosurgery 34 (1994) 1058–1064.
[13] P. Menei, M.C. Venier, E. Gamelin, J.P. Saint-Andre, G. Hayek, E. Jadaud, D. Fournier, P. Mercier, G. Guy, J.P. Benoit, Local and sustained delivery of 5-fluorouracil from biodegradable microspheres for the radiosensitization of glioblastoma: a pilot study, Cancer 86 (1999) 325–330.
[14] J.P. Benoit, N. Faisant, M.C. Venier-Julienne, P. Menei, Development of microspheres for neurological disorders: from basics to clinical applications, J. Control. Release 65 (2000) 285–296.
[15] N. Bru-Magniez, C. de Cock, J. Poupaert, J.L. de Keyser, P. Dumont, Process for the preparation of monoesters or diesters of 9,10-endoethano-9,10-dihydroanthracene-11,11-dicarboxylic acid, novel monoesters or diesters prepared by this process and use thereof for the preparation of symmetrical or asymmetrical methylidene-malonates, U.S. Patent 4,931,584 (1990).
[16] F. Lescure, C. Zimmer, D. Roy, J.M. Teulon, P. Couvreur, Synthesis and evaluation of a new biodegradable monomer, Proc. Control. Release Soc. 18 (1991) 325–326.
[17] P. Menei, V. Daniel, C. Montero-Menei, M. Brouillard, A. Pouplard-Barthelaix, J.P. Benoit, Biodegradation and brain tissue reaction to poly(D,L-lactide-co-glycolide) microspheres, Biomaterials 14 (1993) 470–478.
[18] E. Fournier, C. Passirani, C.N. Montero-Menei, J.P. Benoit, Biocompatibility of implantable synthetic polymeric drug carriers: focus on brain biocompatibility, Biomaterials 24 (2003) 3311–3331.
[19] F. Lescure, C. Seguin, P. Breton, P. Bourrinet, D. Roy, P. Couvreur, Preparation and characterization of novel poly(methylidene malonate 2.1.2.)-made nanoparticles, Pharm. Res. 11 (1994) 1270–1277.
[20] C. Le Visage, F. Quaglia, M. Dreux, S. Ounnar, P. Breton, N. Bru, P. Couvreur, E. Fattal, Novel microparticulate system made of poly(methylidene malonate 2.1.2), Biomaterials 22 (2001) 2229–2238.
[21] A. Geze, M.C. Venier-Julienne, P. Saulnier, P. Varlet, C. Daumas-Duport, P. Devauchelle, J.P. Benoit, Modulated release of IdUrd from poly(D,L-lactide-co-glycolide) microspheres by addition of poly(D,L-lactide) oligomers, J. Control. Release 58 (1999) 311–322.
[22] N. Faisant, J.P. Benoit, P. Menei, Utilisation de microsphères biodégradables libérant un agent anticancéreux pour le traitement du glioblastome, Eur. Patent 1053746A1 (2000).
[23] J.M. Péan, M.C. Venier-Julienne, F. Boury, P. Menei, B. Denizot, J.P. Benoit, NGF release from poly(D,L-lactide-co-glycolide) microspheres. Effect of some formulation parameters on encapsulated NGF stability, J. Control. Release 56 (1998) 175–187.
[24] D. Mathieu, J. Nony, R. Phan-Tan-Luu, New Efficient Methodology for Research Using Optimal Design (NEMROD) Software, LPRAI, Marseille, 1998.
[25] G.A. Lewis, D. Mathieu, R. Phan-Tan-Luu, Statistical and mathematical tools. Introduction to multi-linear regression and analysis of variance, Pharmaceutical Experimental Design, Marcel Dekker, New York, 1999, pp. 151–184.
[26] L.K. Chiu, W.J. Chiu, Y.-L. Cheng, Effects of polymer degradation on drug release: a mechanistic study of morphology and transport properties in 50:50 poly(dl-lactide-co-glycolide), Int. J. Pharm. 126 (1995) 169–178.
[27] G. Spenlehauer, M. Vert, J.P. Benoit, F. Chabot, M. Veillard, Biodegradable cisplatin microspheres prepared by the solvent evaporation method: morphology and release characteristics, J. Control. Release 7 (1988) 217–229.
[28] M.J. Alonso, S. Cohen, T.G. Park, R.K. Gupta, G.R. Siber, R. Langer, Determinants of release rate of tetanus vaccine from polyester microspheres, Pharm. Res. 10 (1993) 945–953.
[29] H.-K. Sah, R. Toddywala, Y.W. Chien, The influence of biodegradable microcapsule formulations on the controlled release of a protein, J. Control. Release 30 (1994) 201–211.
[30] M. Boisdron-Celle, P. Menei, J.P. Benoit, Preparation and characterization of 5-fluorouracil-loaded microparticles as biodegradable anticancer drug carriers, J. Pharm. Pharmacol. 47 (1995) 108–114.
[31] R. Ghaderi, C. Sturesson, J. Carlfors, Effect of preparative parameters on the characteristics of poly(D,L-lactide-co-glycolide) microspheres made by the double emulsion method, Int. J. Pharm. 141 (1996) 205–216.
[32] M.J. Blanco-Prieto, E. Leo, F. Delie, A. Gulik, P. Couvreur, E. Fattal, Study of the influence of several stabilizing agents on the entrapment

and in vitro release of pBC 264 from poly(lactide-co-glycolide) microspheres prepared by a W/O/W solvent evaporation method, Pharm. Res. 13 (1996) 1127–1129.

[33] R. Jalil, J.R. Nixon, Microencapsulation using poly(DL-lactic acid). I. Effect of preparative variables on the microcapsule characteristics and release kinetics, J. Microencapsul. 7 (1990) 229–244.

[34] Y.Q. Hu, J.X. Guo, L.J. Wang, R. Tan, L.Y. Zhen, Preparation and evaluation of insulin-loaded polylactide microspheres using factorial design, Drug Dev. Ind. Pharm. 26 (2000) 1309–1313.

[35] M. Hussain, G. Beale, M. Hughes, S. Akhtar, Co-delivery of an antisense oligonucleotide and 5-fluorouracil using sustained release poly (lactide-co-glycolide) microsphere formulations for potential combination therapy in cancer, Int. J. Pharm. 234 (2002) 129–138.

[36] C. Sturesson, P. Artursson, R. Ghaderi, K. Johansen, A. Mirazimi, I. Uhnoo, L. Svensson, A.C. Albertsson, J. Carlfors, Encapsulation of rotavirus into poly(lactide-co-glycolide) microspheres, J. Control. Release 59 (1999) 377–389.

[37] X.M. Deng, X.H. Li, M.L. Yuan, C.D. Xiong, Z.T. Huang, W.X. Jia, Y.H. Zhang, Optimization of preparative conditions for poly-DL-lactide-polyethylene glycol microspheres with entrapped Vibrio cholera antigens, J. Control. Release 58 (1999) 123–131.

[38] M.I. Ugwoke, R. Kinget, Influence of processing variables on the properties of gelatin microspheres prepared by the emulsification solvent extraction technique, J. Microencapsul. 15 (1998) 273–281.

[39] M.B. Sintzel, A. Merkli, C. Tabatabay, R. Gurny, Influence of irradiation sterilization on polymers used as drug carriers: a review, Drug Dev. Ind. Pharm. 23 (1997) 857–878.

[40] E. Fournier, C. Passirani, C. Montero-Menei, N. Colin, P. Breton, S. Sagodira, P. Menei, J.P. Benoit, Therapeutic effectiveness of novel 5-fluorouracil-loaded poly(methylidene malonate 2.1.2)-based microspheres on F98 glioma-bearing rats, Cancer 97 (2003) 2822–2829.