Preparation and Characterization of Novel Poly(methylidene Malonate 2.1.2.)-Made Nanoparticles

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Received December 13, 1993; accepted March 24, 1994

Poly(methylidene malonate 2.1.2.) (PMM 2.1.2.) nanoparticles were prepared in phosphate buffer through emulsion polymerization of monomeric units; the kinetics of the reaction was monitored by spectrophotometry at 400 nm. Average nanoparticle sizes, molecular weights, and biodegradability of this potential drug carrier were determined under various conditions. As previously demonstrated for other similar monomers, *i.e.* IHCA or IBCA, pH influenced the physico-chemical characteristics of the nanoparticles obtained. Ethanol release from the ester-bearing side chains indicated that the polymers were susceptible to hydrolysis when incubated in basic pH or in rat plasma. A secondary degradation pathway, yielding formaldehyde through a reverse Knoevenagel's reaction, was minimal. Cytotoxicity studies of this new vector, *in vitro,* against L929 fibroblast cells demonstrated that PMM 2.1.2. nanoparticles were better tolerated than other poly(alkylcyanoacrylate) (PACA) carriers. Pharmacokinetic studies were also carried out to observe the fate of ^{14}C-labelled PMM 2.1.2. nanoparticles after intravenous administration to rats. Forty eight hour post-injection, more than 80% of the radioactivity was recovered in urine and faeces. The body distribution of the polymer was estimated by measuring the radioactivity associated with liver, spleen, lung and kidneys. Five minutes after injection, a maximum of 24 ± 2% of the total radioactivity was detected in the liver and less than 0.4% in the spleen. The liver-associated radioactivity decreased according to a biphasic profile and less than 8% of the total radioactivity remained after 6 days.

KEY WORDS: drug targeting; polymeric drug carrier; nanoparticle; polymerization; biodegradation; pharmacokinetics.

INTRODUCTION

Submicronic colloidal polymeric particles (nanoparticles) have been proposed as drug carriers to modify the pharmacokinetic and tissue distribution profile of biologically active compounds. Site selective drug delivery with nanoparticles was successful in the treatment of some murine experimental neoplasic diseases [1] and in improving the intracellular diffusion of antimicrobial agents [2]. Furthermore, the ability of nanoparticles to protect a drug from rapid metabolism and to improve its diffusion through biological barriers was demonstrated for peptide delivery [3, 4].

Among the synthetic polymeric colloidal drug carriers available, poly(alkyl-2-cyanoacrylate) (PACA) nanoparticles [5] are one of the most advanced polymeric colloidal systems for pharmaceutical applications. For instance, a phase I clinical trial, using doxorubicin-loaded PACA nanoparticles, in 21 patients with refractory solid tumors revealed an improvement of the free doxorubicin therapeutic index [6]. Some significant advantages of alkylcyanoacrylate over other derivatives also used for preparing nanoparticles are the anionic polymerization process occurring in water [7] and their biodegradation through enzymatic hydrolysis of the ester functions [8].

However, some PACA properties, such as the low polymerization pHs (around 2) and some *in vitro* cytotoxicity [9] represent major drawbacks. This led to the synthesis of new dialkyl-methylidene malonic acid esters monomers [10]. Recently, poly(diethylmethylidene malonate) (PDEMM) nanoparticles were prepared, but were shown to be non-biodegradable both *in vitro* and *in vivo* [11]. Since ester hydrolysis is considered as the limiting step in biodegradation, the difference in the behavior of PACA and PDEMM was accounted for by stabilization of the transition state leading to the tetrahedral adduct intermediate generated in the hydrolysis [11]. Indeed, the cyano group is a stronger electron-captor substituent than the alkoxycarbonyl group, and consequently, a better stabilizing group.

This paper describes a new methylidene malonate derivative, *i.e.* ethyl-2-ethoxycarbonylmethylenoxycarbonyl acrylate (MM 2.1.2.) which was designed to overcome the lack of degradation encountered with PDEMM. Preparation and characterization of poly(MM 2.1.2.) (PMM 2.1.2.) nanoparticles by anionic polymerization are presented together with data on biodegradation mechanisms and *in vivo* fate.

MATERIALS AND METHODS

Chemicals

1-Ethoxycarbonyl-1-ethoxycarbonylmethylenoxycarbonyl ethene monomer (M_w = 230) also referred to as methylidene malonate 2.1.2. (MM 2.1.2.) was prepared in UPSA Laboratories / Carpibem (Rueil Malmaison, France) according to Bru-Magniez *et al.* [12]. The 1-^{14}C labelled counterpart (specific activity : 1.13 $mCi.mmol^{-1}$) was from Isotopchim (Ganagobie - Peyrueis, France). Sufficient amount of sulphur dioxide (SO_2) was added to prevent polymerization process. Dextran (M_w : 70,000) was purchased from Fluka Chemie AG (Buchs, Switzerland). Most of the buffering salts, reactants and solvents were from Prolabo (Paris, France). All products for tissue culture were provided by Gibco (Cergy-Pontoise, France).

Cells

In vitro cytotoxicity experiments were carried out on murine L929 fibroblast cell line (Flow, les Ulis, France). Cells were maintained in MEM medium containing foetal bovine serum (5 %), L-Glutamine (2 mM), penicillin (50

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μg.mL^{-1} and streptomycin (50 μg.mL^{-1}) and were split every 3-4 days after a 10 min.-treatment with a 0.25 % trypsin - 0.02% EDTA mixture at 37°C.

Animals

Pharmacological experiments, including organ distribution of ^{14}C-labelled MM 2.1.2. nanoparticles and radioactivity recovery into urines and faeces, were performed on male Sprague-Dawley rats (Charles Rivers, Saint-Aubin-les-Elboeuf, France) weighting between 120 and 150 g. Rodents were distributed in cages as follows: 3 rats per cage for organ uptake (one cage per time of observation) and one per cage for the excretion statement [13].

Nanoparticle Preparation and Characterization

Poly(methylidene malonate 2.1.2.) nanoparticles were prepared, under sterile conditions, through anionic polymerization in phosphate buffer (KH_2PO_4/Na_2HPO_4 0,066M, pH 5.5) supplemented with 1 % dextran [14]. Briefly, 100 μL of monomer rendered SO_2-free (3 h, 10^{-2} Torr) were added dropwise under magnetic stirring to 10 mL of polymerization medium maintained at 25°C. Particle formation was followed by measuring suspension turbidity on a Model 25 spectrophotometer (Beckman, Gagny, France) at 400 nm. After 24 hours polymerization, the nanoparticle suspension was filtered on 8 μm filter paper (Whatman, Maidstone, England). Nanoparticles were subsequently freeze-dried and thus, could be stored for long periods of time at room temperature. They were generally resuspended just before use by adding an appropriate volume of distilled water to obtain an effective concentration of 6 to 8 mg of polymer per mL. Nanoparticle size was measured as follows: Thirty seven point five microliters of nanoparticle suspension were diluted in 3 mL water contained in a plastic cuvette (1 cm × 1 cm) which was then placed in the stage of a submicrometer particle sizer Model N4 (Coulter Electronics, Hialeah, FL). The constitutive polymer molecular weight distribution was estimated by size exclusion chromatography (SEC) (Polymer Laboratories, Church Stretton, England) as described elsewhere [15]. Polymerization yield was calculated by pelleting (120,000 g for 5 min.), drying and finally weighing the solid residue isolated from the initial suspension. PIHCA and PIBCA nanoparticles were prepared according to Couvreur *et al.* [7].

PMM 2.1.2. Nanoparticle Degradation Product Assay

Degradation of PMM 2.1.2. nanoparticles was assessed by measuring the amount of ethanol released (Sigma Kit 330-1). Another degradation pathway, leading to the formation of formaldehyde was also evaluated [8]. The presence of formaldehyde in PMM 2.1.2. nanoparticle suspension was observed by using a Hantzsch's reaction-based colorimetric assay [16]. Briefly, acetylaceton reacts with formaldehyde in presence of ammonia to yield 3,5-diacetyl-1,4-dihydrobutidine which can be spectrophotometrically measured at 415 nm.

Kinetic of PMM 2.1.2. Nanoparticle Degradation

Ethanol production was measured at different pH values by means of the assay mentioned above. Two milliliters of PMM 2.1.2. nanoparticle suspension were suspended in 4 mL of buffers at various pHs, sealed in ampoules to avoid any loss of ethanol, and incubated at 37°C for different periods of time. The suspension was then pelleted (120,000 g for 5 min.) and ethanol released was measured in the supernatant. Similarly, formaldehyde production was followed at various pHs. Briefly, one milliliter of nanoparticle suspension (6-8 mg polymer per mL) was added to 3 mL of buffer A, B, C or D (A = KCl/HCl 0.2M pH 2.0 ; B and C = Citrate/Phosphate 0.2M pH 5.5 and 7.2 ; D = Glycine-NaCl/HCl 1M pH 9.0) and kept at 37°C. At different times, 0.2 mL of suspension was mixed with 0.8 mL of phosphate buffer 0.066M, pH 7.4 and 2 mL of Nash's reactant consisting in 150 g ammonium acetate, 3 mL acetic acid and 4 mL acetylacetone (Merck, Darmstadt, Germany) in 1 L water [16]. Hantzsch's reaction took place for 1 hour at 37°C and OD_{415nm} was then measured. The plasmatic degradation was carried out in measuring ethanol release ; one volume of nanoparticles being incubated in two volumes of rat plasma at 37°C for 4, 8, 24, 32 and 48 hours.

In Vitro Cytotoxicity

Fibroblast L929 cells were distributed into 96-well plates at 1.25 × 10^4 cells per mL of culture medium (200 μl per well), and incubated for 24 hours at 37°C in a humidified and CO_2-enriched (5 %) atmosphere, to allow adherence and proliferation. At that time, wells were drained off and different amounts of nanoparticles (PMM 2.1.2., PIHCA or PIBCA), degraded PMM 2.1.2. nanoparticles, ethanol or glycolic acid contained in complete MEM were added for a 24 or 48 hour-incubation period at 37°C in the same conditions as described above. Cytotoxicity was determined according to the methodology published by Borenfreund and Puerner [17]. Briefly, culture supernatant was eliminated and cells washed twice with washing medium (culture medium supplemented with 20 mM HEPES) prior treatment with 200 μl per well of neutral red solution (50 μg per mL of washing medium) to ensure dye uptake exclusively into viable cells. Three hours later, neutral red was removed and cells were further fixed with a solution of formaldehyde (3.7 %) and $CaCl_2$ (1 %) (100 μL per well) for 1 min., then homogenized in a water/ethanol/acetic acid mixture (49.5/49.5/1.0 - v/v/v) (200 μL per well). Optical density of the resulting solution was read at 540 nm on a EL 310 Bio-tek Instruments microplate reader (Winooski, VT).

Accordingly, the percentage of cytotoxicity was calculated as following:

$$\text{Cell viability (\%)} = \frac{OD_{sample}}{OD_{control}} 100$$

$OD_{control}$ was determined from untreated L929 cells.

Pharmacokinetic Studies

Each unfed rat received one single dose of approximately 6 mg of radioactive nanoparticles, resuspended in 0.3% NaCl and injected intravenously (3 mL . 50 μCi.kg^{-1} body weight) in bolus through the caudal vein. Urines and faeces of rats were then carefully collected at different peri-

ods of time (minimum 3 rats per time). An aliquot of each urine fraction (ca 0.2 g) was counted after adding 10 mL of Quicksafe A scintillant fluid (Zinsser Analytic, Maidenhead, England). Faeces were vigorously blended with three times their weight of water and 0.1 g of this mixture was digested with 1 mL Lumasolve (Lumac, Landgraaf, Netherlands) at 50°C for 4 to 5 hours, followed by addition of 15 mL Quicksafe A/HCl 1N (9/1 ; v/v). Similarly, organs were digested in 5N KOH for 24 hours at 50°C; aliquots (0.2 g) of each sample were counted after adding 15 mL Quicksafe A/HCl 1N (9/1 ; v/v). Radioactivity was measured on a Beckman LS 1701 liquid scintillation counter.

RESULTS

Nanoparticle Preparation and Characterization

Anionic polymerization of MM 2.1.2. occurred in aqueous medium, leading to nanoparticle suspensions with characteristics mainly depending on the pH of the polymerization medium. In the pH range from 5.0 to 6.0., the mean nanoparticle size decreased from 450 to 250 nm with an excellent batch to batch reproducibility; the smaller particles being obtained above pH 5.5 (Fig. 1). Nanoparticles could not be obtained at lower pHs. At higher pHs, proton concentration did not significantly modify the nanoparticle volume and polymerization generated nanoparticles with sizes ranging from 200 to 500 nm, with poor yields, along with many sticky aggregates. In all cases, polymer molecular weight of the resulting solid phase (nanoparticles or aggregates) were dramatically affected by the pH of the polymerization medium (Fig. 1). A minimum in the weight average molecular weight M_w was observed at pH 5.5, with values calculated around 1000-2000 eq. polystyrene. Above pH 5.5, M_w constantly increased up to approximately 60,000 to 70,000 (eq. polystyrene) at pH 9.0. Experimentally, the best conditions for nanoparticle preparation was pH 5.5 where both the yield (amount of monomer converted into polymer) and nanoparticle size were satisfactory and reproducible.

Kinetics of nanoparticle formation at pH 5.5, followed by turbidity and size measurements, was shown to increase during the first 5 hours, then reached a plateau during the next 19 hours (Fig. 2). SEC profiles showed that molecular weights evolved with time towards a priviledged species (Fig. 3). All of the nanoparticle characteristics, such as size, yield, molecular weights and turbidity were no longer changing after 24 hours. These conditions (pH 5.5 and 24 hours polymerization) were chosen as standard in subsequent batches for *in vitro* or *in vivo* experiments.

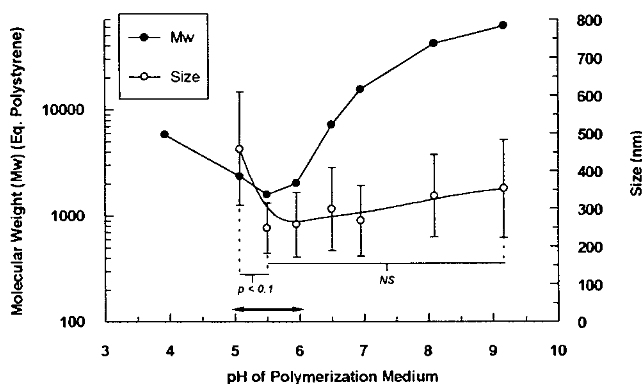

Fig. 1. Influence of the pH on both PMM 2.1.2. molecular weight (M_w) (mean, n = 3) and size of resulting nanoparticles (mean ± SD, n = 5). Measures were performed as mentioned in "Materials and Methods". The arrow delimits the pH range within which polymerization yielded nanoparticles with reproducible characteristics. *NS* = Not Significant.

In Vitro Assessment of Nanoparticle Degradation

Degradation of MM 2.1.2. nanoparticles was assessed by measuring ethanol (Fig. 4) and formaldehyde production, at different pHs in the absence of enzymes. Contribution of the formaldehyde pathway to the degradation of PMM 2.1.2. was low for all pH tested. Indeed, the amount of formaldehyde produced in 24 hours, even at pH 9.0, was less than 4.5% of the maximum theoretical amount produced if the polymers were entirely degraded through this route (Data not shown). The degradation process, via ester hydrolysis of the polymer side chains, led to ethanol release (Fig. 5). Within 24 h, degradation of the polymer through ester breaking was prevalent at basic pH (9.5 and above), following first-order kinetics. Beyond 24 h, ethanol release reached a plateau (a maximum of 44% ethanol was released after 144 h incubation at pH 11.5). At pH 7.0 and 8.5, hydrolysis was significantly reduced and it was absent at a polymerization pH of 5.5. Finally, under biological conditions, in rat plasma, the PMM 2.1.2. nanoparticles were highly sensitive to ester hydrolysis, especially because of the presence of esterases with a maximum of 25% ethanol released within the first 24 h.

Cytotoxicity Studies

Cytotoxicity of PMM 2.1.2 nanoparticles was determined on fibroblast L929. The 50% of cell viability (LD_{50}) occurred at concentrations of 30 and 50 $\mu g.mL^{-1}$ after 1 and 2 days incubation, respectively, compared to 12 $\mu g.mL^{-1}$ for poly(isohexylcyanoacrylate) and to 8 $\mu g.mL^{-1}$ for poly(isobutylcyanoacrylate) after day 1 or 2 (Fig. 6). At 30

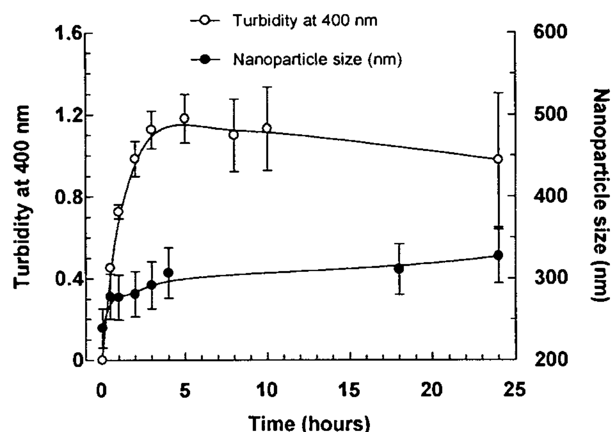

Fig. 2. Kinetic of polymerization and nanoparticle formation, monitored by measuring the variation of nanoparticle sizes and turbidity at 400 nm (mean ± SD, n = 5). Nanoparticle diameters were measured as in Figure 1. Turbidity values were obtained from a 27-fold water diluted nanoparticle suspension introduced in a plastic cuvette (1 cm × 1 cm) held in the stage of a spectrophotometer.

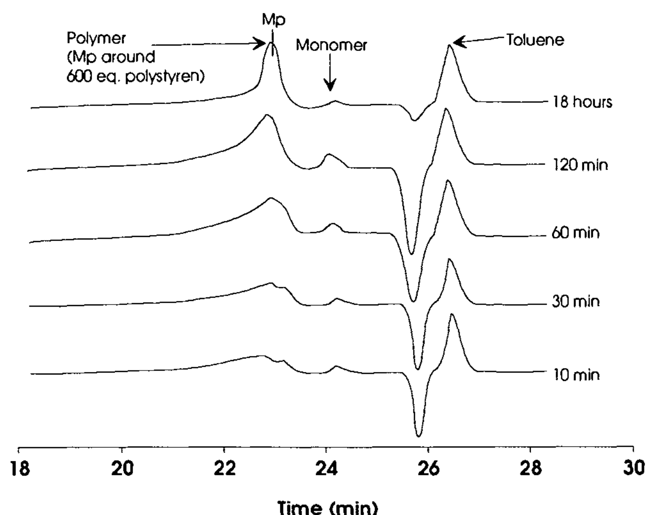

Fig. 3. Size exclusion chromatography profiles for different time of polymerization showing the emergence of a polymeric species at approx. M_p = 600 in equivalent polystyrene. Experiments were performed at different indicated times as mentioned in "Materials and Methods." Detection was made by refractometry.

$\mu g.mL^{-1}$, the predegraded nanoparticles had no effect on cell viability ; total cell death appeared only for doses as high as 400 $\mu g.mL^{-1}$. The poor cytotoxicity of PMM 2.1.2. metabolization products was also confirmed after incubation of cells with ethanol and glycolic acid which are two of the proposed metabolites. These compounds were found to be non-cytotoxic at the tested doses.

Fate of PMM 2.1.2. Nanoparticles after Intravenous Administration

After intravenous administration of PMM 2.1.2. nanoparticles, radioactivity was recovered in both urine and faeces. 76% of the total radioactivity was eliminated during the first day and 86% within 6 days. Faecal elimination was more extensive than urinary excretion (Table I). Kinetics of urinary excretion were biphasic (Fig. 7). Five minutes after injection, 24% of the total radioactivity were found in the liver and 0.4% in the spleen, which represented 4.76 and 0.61% per gram of organs respectively (Fig. 8). At the end of the study (6 days), 8% of the total radioactivity still persisted in the liver and 0.15% in the spleen (respectively 0.71 and 0.23% per gram of organs). Radioactivity was also found in lungs and kidneys but most was eliminated from these organs within 24 hours (Fig. 8).

DISCUSSION

Nanoparticles were prepared by anionic polymerization of MM 2.1.2. monomers in aqueous medium containing dextran. The pH value of the polymerization buffer critically influenced the physico-chemical characteristics of the nanoparticles, such as size and molecular weight of the constitutive polymers. The plot of size versus pH (Fig. 1) was consistent with those reported previously by Douglas *et al.* [18] for poly(butyl-2-cyanoacrylate) (PBCA) and by De Keyser *et al.* [11] for poly(diethyl methylidene malonate) (PDEMM) nanoparticles. However, in the present analysis, minimum size was achieved at pH 5.5 - 6.0, in comparison to pH 2.0 and pH 7.6 for PBCA and PDEMM, respectively.

As shown previously for poly(alkylcyanoacrylate) (PACA) [15, 19-21], the weight-average molecular weight of PMM 2.1.2. was sensitive to pH variations (Fig. 1). Below pH 5.0, MM 2.1.2. monomers polymerized but almost no nanoparticles were formed. From pH 4.0 to 5.5, the molecular weight (M_w) dramatically decreased and approached, between pH 5.5 and 6.0, a monodisperse polymer, with a minimal value of 1500 (M_w). In the pH range 5.5 - 6.0, these polymers led to the smallest nanoparticles (~250 nm) along with the best yields. Unlike PACA nanoparticles [19, 20], no correlation could be established between PMM 2.1.2. weight average molecular weight and size of corresponding nanoparticles. Douglas *et al.* [21] suggested that the effect of polymerization pH is complex and, molecular weights depend on the poorly understood antagonistic action of initiator (OH^-) and terminator (H^+) of the polymerization process. The main goal of the present experiments was to establish the best pH conditions for the preparation of PMM 2.1.2. nanoparticles. Based on particle size and yield, the pH range 5.5 - 6.0 clearly was optimal. Moreover, for these pH values, the low molecular weight was essential to favorable bioelimination kinetics and degradation rate [22].

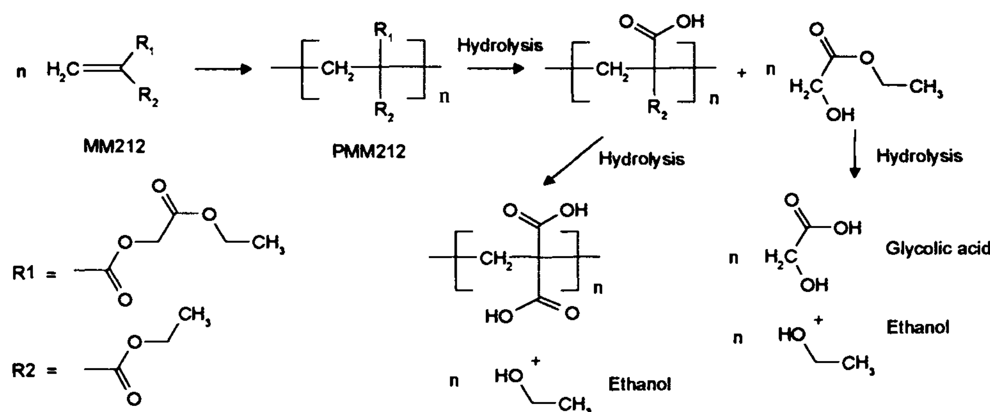

Fig. 4. Degradation pathway of PMM 2.1.2 side chains. The potential backbone degradation process occuring through a reverse Knoevenagel's reaction is similar to that detailed for poly(alkyl α-cyanoacrylates) by Leonard *et al.* [23].

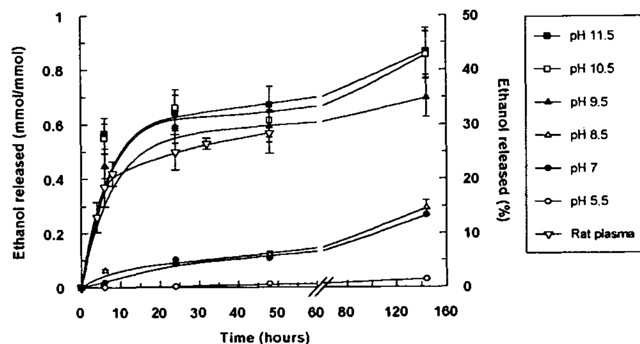

Fig. 5. Kinetic of PMM 2.1.2. nanoparticle degradation through ester hydrolysis leading to ethanol release; profiles were obtained for various pH conditions and in rat plasma according to the methodology described in "Materials and Methods" (mean ± SD, n = 3).

The preferred time to obtain a full polymerization reaction was the other important parameter which needed to be controlled. Polymerization kinetics followed by turbidity and size measurements (Fig. 2) demonstrated that nanoparticle formation occurred within 5 hours at pH 5.5. The slight decrease in turbidity between 5 and 24 hours could be attributed to bioerosion of the nanoparticles inducing PMM 2.1.2. solubilization. Size exclusion chromatography (SEC) (Fig. 3), demonstrated the emergence of polymerization reaction products only during the first two hours. Based on these observations, nanoparticle formation was allowed to proceed for 22 h.

Table I. Summary of the percentages of urinary and faecal excretion of radioactive PMM 2.1.2. nanoparticles after I.V. injections to rats

	Time period		
	0–24 h	0–48 h	0–144 h
Faeces	43 ± 5	46 ± 6	48 ± 6
Urines	33 ± 11	35 ± 11	38 ± 11
Total	76 ± 12	81 ± 12	86 ± 12

The biodegradability of PMM 2.1.2. was important to ensure bioelimination of the nanoparticles. Based on previously synthesized non-degradable PDEMM nanoparticles [11] and on the degradation pathway of PIBCA described by Lenaerts *et al.* [8], our strategy was to introduce a labile ester of ester function into the methylidene malonate series to yield a degradable polymer, i.e., PMM 2.1.2.. As ex-

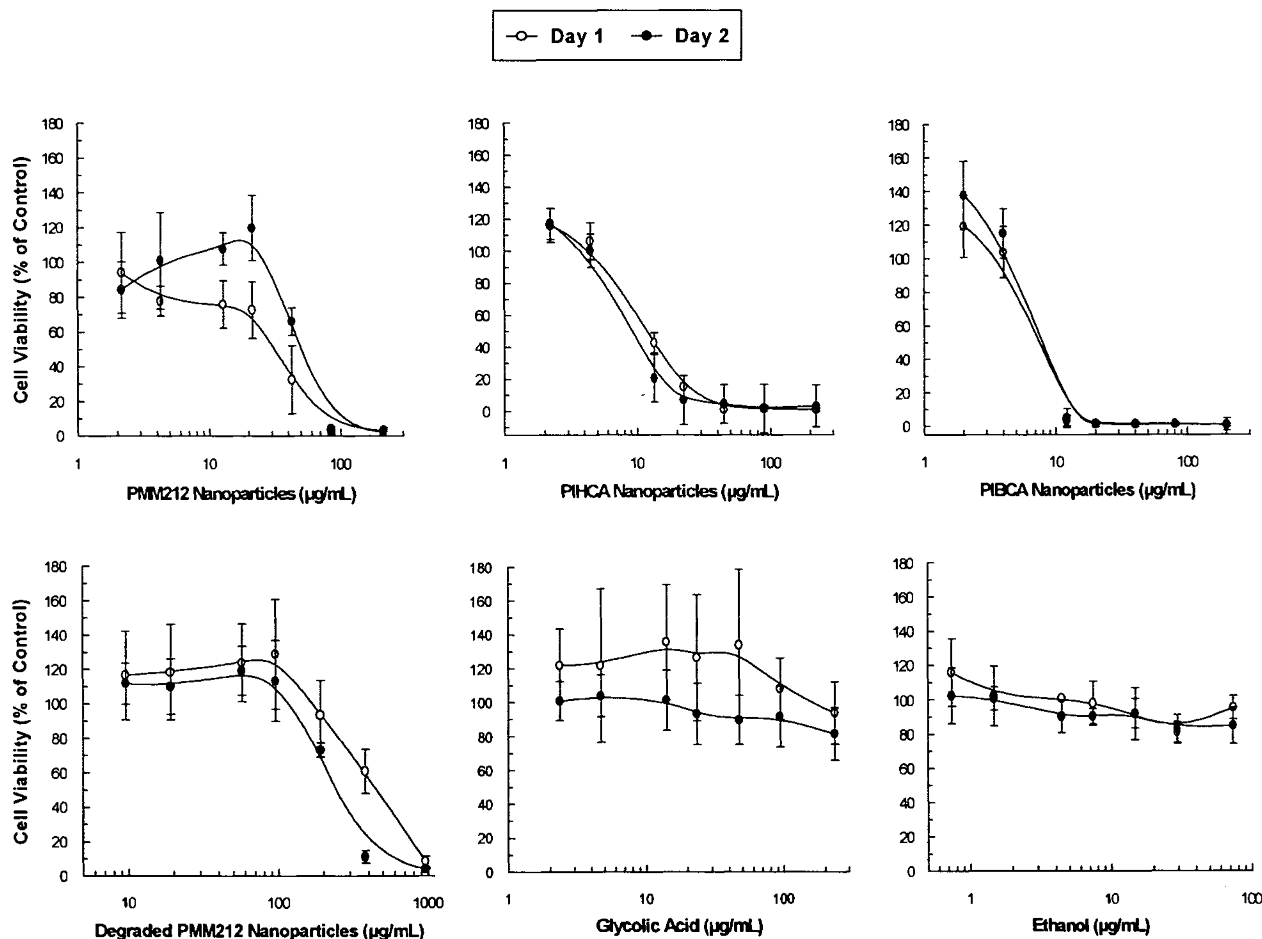

Fig. 6. *In vitro* cytotoxicity of PMM 2.1.2. nanoparticles and its degradation products on L929 fibroblasts. For comparison, PIHCA and PIBCA nanoparticle toxicities are also shown. Cells were incubated with each component for 24h (Day 1) and 48h (Day 2). Methodology employed was detailed in "Materials and Methods" (mean ± SD, n = 4).

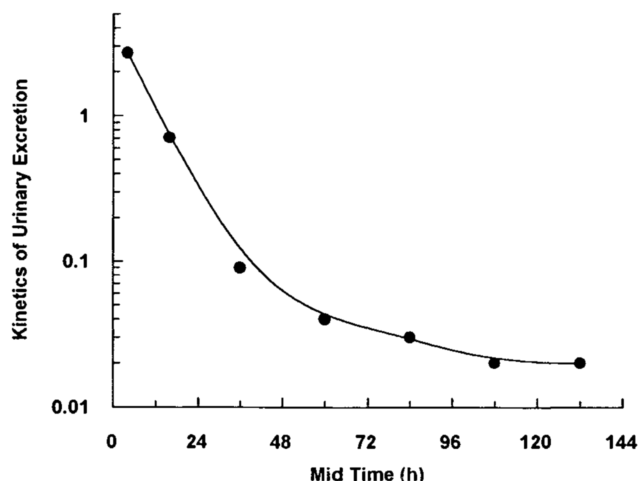

Fig. 7. Kinetic of radioactivity urinary excretion after intravenous administration of a single dose of ^{14}C-radiolabeled PMM 2.1.2. nanoparticles (see "Materials and Methods") (mean, n = 3 per time).

pected, at basic pHs and in rat plasma, PMM 2.1.2. nanoparticles were degraded into soluble polymers, glycolic acid and ethanol (Figs. 4 and 5). The mechanism postulated by Lenaerts *et al.* [8] can be assumed to apply, through ester saponification at low proton concentration or enzymatic hydrolysis by plasmatic esterases. Erosion and enzymatic bioerosion were suggested to occur at the nanoparticle surface, mainly because of ester hydrolysis which generates free carboxyl groups and leads to soluble polymers. In aqueous solution above pH 7.0, the hydroxyl anions were also reported to attack the polymeric chain leading to formaldehyde release as one of the degradation products [22-24]. As already demonstrated several years ago for PACA [8, 25], the degradation of the polymer backbone occurring through the reverse Knoevenagel's reaction was a minor degradation pathway, since the production of formaldehyde did not exceed 1% after 24 h incubation at pH 5.5 and 3.6% at neutral pH.

In vitro cytotoxicity (Fig.6) and pharmacokinetics (Table I, Fig. 7) of PMM 2.1.2. nanoparticles were also studied to evaluate the potential of this new drug carrier. As previously done with PACA nanoparticles [9], studies were performed to measure the PMM 2.1.2. nanoparticle *in vitro* cytotoxicity in L929 fibroblast cell culture. Poly(methylidene malonate 2.1.2.) nanoparticles were three and five time less toxic than PIHCA and PIBCA nanoparticles, respectively (based on LD_{50} values). Lherm *et al.* [9] proposed a mechanism to account for PACA nanoparticle cytotoxicity, by which particle adhesion to L929 cells could represent the

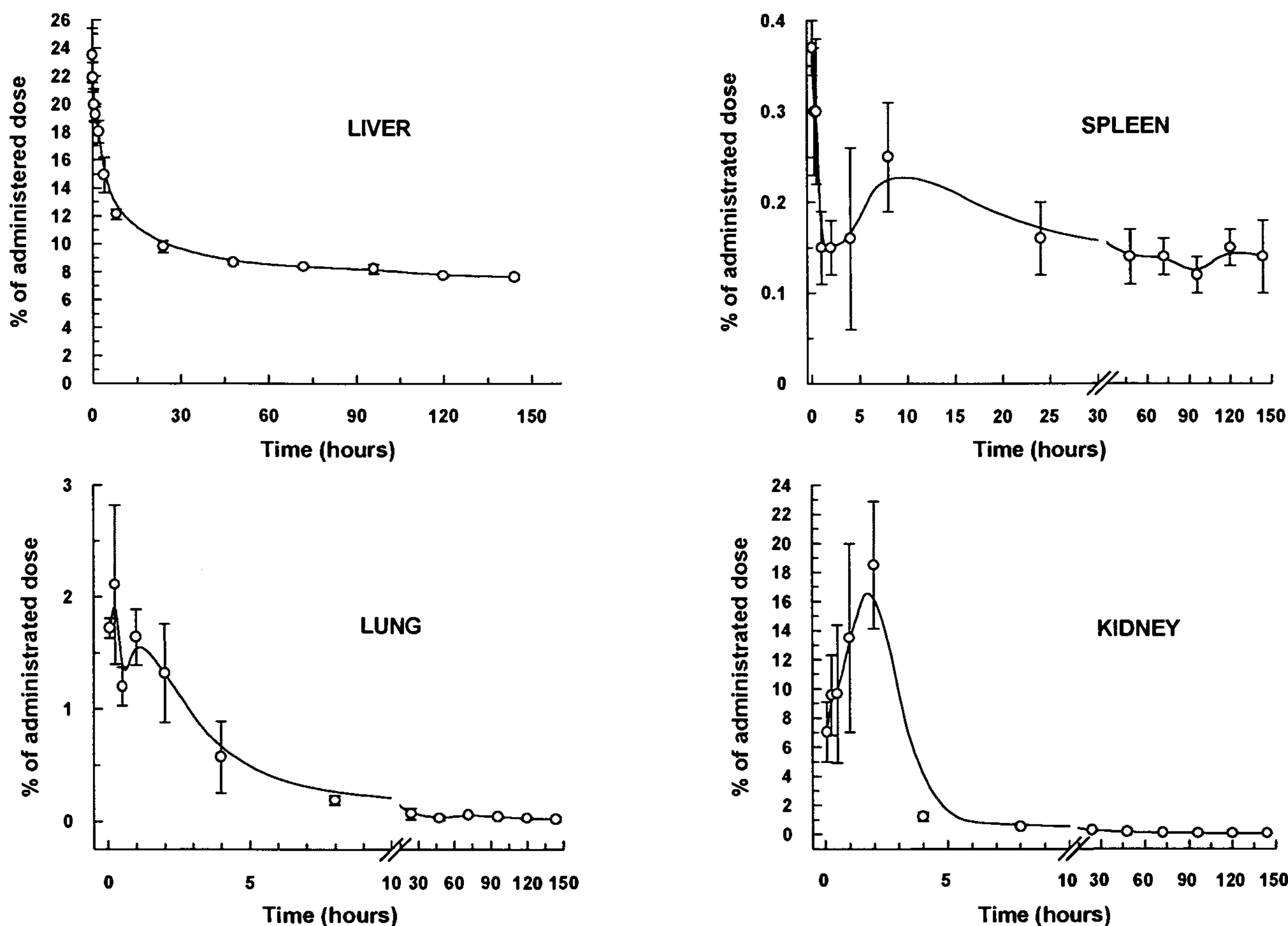

Fig. 8. Distribution of radioactivity in various organs, after intravenous administration of a single dose ^{14}C-radiolabelled PMM 2.1.2. nanoparticles (see "Materials and Methods") (mean % of dose ± SD, n = 3 per time).

initial step of the process. According to these authors, cytotoxicity of these colloidal polymeric carriers is closely related to their degradation occurring near the cell surface, thus generating high local concentrations of degradation products responsible for cell damage. This mechanism could apply for PMM 2.1.2. nanoparticles, with faster degradation kinetics than those observed for PACA and especially for PIHCA (the faster the degradation, the less the cytotoxicity) [9]. The lower cytotoxicity might also be due to differences in surface properties, because PMM 2.1.2. nanoparticles were shown to adhere weakly to the cell surface, as confirmed in experiments with radiolabelled PMM 2.1.2. (data not shown), where the percentage of radioactivity associated with cells was approximately 1%. In addition, two of the degradation products (glycolic acid and ethanol) appeared to be non-toxic up to 100 μg per mL of cell suspension (higher doses than the maximum theoritical amounts released). In these experiments, glycolic acid could even be described as a cell growth stimulator.

Determination of PMM 2.1.2. molecular weight (M_w) was essential to allow a correct evaluation concerning the risk of *in vivo* polymeric overloading resulting in cytotoxicity. A polymer with a molecular weight higher than 50,000 would be retained by glomerular filtration even if solubilized through ester function hydrolysis. Thus, nanoparticles formed by PMM 2.1.2. with low molecular weights were expected to be excreted. This was observed after intravenous administration of ^{14}C-radiolabeled PMM 2.1.2. nanoparticles : more than 80 % of the radioactivity was eliminated within 48 hours and, for all organs tested, radioactivity decreased with time. In addition, PMM 2.1.2. nanoparticles were observed to be less extensively captured by the mononuclear phagocytic system than polystyrene [26], polymethylmetacrylate [27], or polyalkylcyanoacrylate nanoparticles [28, 29]. Shortly after injection, the liver uptake was only 25 % with PMM 2.1.2. nanoparticles whereas it was above 50 % with the other types of nanoparticles. As a consequence, PMM 2.1.2. nanoparticles were removed less rapidly from the circulation ($T_{1/2\alpha}$ was 100 min compared to 5 min for polyisobutylcyanocrylate nanoparticles [28]). Further experiments are in progress to see if the reduced liver uptake of PMM 2.1.2. nanoparticles is related to any of its physicochemical properties leading to reduced opsonization and macrophages (i.e. Kupffer cells) capture.

In conclusion, the development of a new monomer, on the same basis as alklycyanoacrylates, led to the synthesis of alkylmethylidene malonates, from which MM 2.1.2. was selected for its physico-chemical characteristics and biological properties. Nanoparticles made of PMM 2.1.2. may be useful for drug targeting.

ACKNOWLEDGMENT

The authors gratefully thanks Dr. J.M. Teulon for providing methylidene malonate 2.1.2. as well as Drs. S. Decourt, P. Hermann, P. Wüthrich and Dr. J.P. Michel for their significant contribution.

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