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Release of a macromolecular drug from alginate-impregnated microspheres

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Abstract

Macroporous microspheres were impregnated with calcium alginate to encapsulate fluorescein isothiocyanate-labeled dextran (FITC-dextran) and control its release. The detailed study of the impregnation process lead to its optimization: the quantity of alginate in the impregnated microspheres and the FITC-dextran encapsulation efficiency were increased. FITC-dextran diffused out of the impregnated microspheres in a slow rate in deionised water, while in presence of sodium ions, its release rate was increased as a consequence of the progressive swelling and erosion of calcium alginate. Release studies from different formulations of impregnated microspheres were performed in a continuous flow apparatus. The release profiles were composed of a slow release phase explained by the progressive erosion of calcium alginate and a faster release phase related to eroded impregnated microspheres. Therefore, the delayed release by microspheres induced by impregnation would permit the delivery of their payload at the vascular occlusion site, limit the amount of drug lost in the systemic circulation and improve the therapy.

Keywords: Chemoembolization; Alginate; Delayed release; Microspheres; Impregnation

1. Introduction

Discovery of new pharmacological targets (Buolamwini, 1999) and new strategies (Jahrsdorfer and Weiner, 2003) in anticancer therapy has promoted the development of novel macromolecular anticancer agents (nucleic acids, peptides or proteins) (Carpentier et al., 2000; Read and Bremner, 2002; Chada et al., 2003). A critical issue remains their poor tissue distribution and their reduced stability in biological fluids. Drug carriers have been developed, using various kinds of technologies, which can be represented in two classes: targeted circulating nanoparticles (Brigger et al., 2002) and intratumoral implants (Saltzman and Fung, 1997). Another promising approach, transarterial chemoembolization, offers various advantages. The occlusion of selective arterial branches reduces

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the blood supply to the tumor. The reduced blood flow inside the tumor increases drug absorption (Johansson, 1996). Several preclinical and clinical studies have successfully introduced drug delivering microspheres designed for embolization in their trials (Benita et al., 1984; Codde et al., 1990; Yang et al., 1995; Fujiwara et al., 2000). These drug delivery systems were designed for the administration of classical low molecular weight anti-cancer drugs (anthracyclines, aziridines, platine derivatives) (De Maio et al., 2003). Therefore, the development of drug delivering microspheres for embolization dedicated to the transport and the controlled release of macromolecular compounds would be relevant.

Our group focuses on the modification of acrylic microspheres suitable for embolization (Beaujeux et al., 1996; Pelage et al., 2003) through impregnation by calcium alginate (Boudy et al., 2002). This method was developed as an alternative to microencapsulation and coating techniques, as modifications in the dimensions, in the shape of the particles and in the surface (Suryakusuma and Jun, 1984), might have dramatic issues on biocompatibility and hemocompatibility (Rihova, 1996). We have recently shown that alginate impregnated in the microspheres controlled the release of a low molecular weight drug, indomethacin, without altering the surface properties of the microspheres (Chretien et al., 2004). Thus, controlled release properties of calcium alginate were transposed to our alginate-impregnated microspheres.

Several macromolecular drug delivery systems are based on calcium alginate (Ho and Neufeld, 2001; Gu et al., 2004). Besides release through diffusion out of the calcium alginate gel, swelling and erosion of the drug delivery system has been described as the major source of release of encapsulated macromolecular drugs (Murata et al., 1993). Correlation between calcium alginate swelling-erosion rate and drug release rate allowed Kikuchi's team to produce a pulsatile drug delivery system, characterized by a controlled lag time before release (Kikuchi et al., 1997).

Once injected, microspheres are drifting in the arterial flow towards the embolization site. Drug delivery by microspheres for embolization is desired to take place at the embolization site. A lag time between microsphere injection and drug release would limit the systemic spill-over of the drug and favor a high drug concentration at the embolization site. Therefore, we have investigated how alginate-impregnated microspheres could transport and delay the release of a macromolecular tracer, fluorescein isothiocyanate-labeled dextran (FITC-dextran). We preliminarily studied the influence of alginate formulation parameters on microsphere agglomeration and on FITC-dextran encapsulation. The optimization of FITC-dextran release conditions motivated the study of its release kinetics from impregnated microspheres in a continuous flow system. Cross interpretation of our results allowed us to determinate the parameters leading to a delayed release of FITC-dextran.

2. Experimental section

2.1. Materials

GF2000-Trisacryl LS microspheres were obtained from BioSepra s.a. (France). This resin, composed of poly *N*-Tris hydroxymethyl methylacrylamide, was originally designed as a chromatographic media for biomacromolecule purification (Brown, French Patent no. 7723223, 1977). Preliminary biocompatibility studies (Laurent et al., 1996; Labarre et al., 2002) validated their choice as an embolization material. These materials are hydrophilic macroporous chromatographic resins prepared as spherical semi-rigid microbeads of 80–160 μm diameter with a mean diameter of 120 μm. The exclusion molecular weight limit of the microspheres is approximately 10^7. Manugel DMB and Keltone HVCR sodium alginate were a gift from ISP (France). Their mannuronate-to-guluronate ratio (M/G) was studied by another group (Wang and Garth, 1998) ($M/G_{Manugel\ DMB} = 1$; $M/G_{Keltone\ HVCR} = 1.5$). FITC-dextran with a molecular weight of 70,000 was obtained from Sigma (France).

2.2. Preparation of impregnated microspheres

The resin was washed by rinsing ~100 mL of wet resin with 5×250 mL volumes of deionised water. Briefly, the resin was suspended by mechanic agitation. After complete settlement, the resin was recovered by vacuum filtration through a 40 μm mesh. Fresh deionised water was added to the resin, which was then freeze-dried.

FITC-Dextran was dissolved in 25 mL of a 1, 2, 3 or 4% (w/v) sodium alginate solution in water to a concentration of 2 mg mL^{-1}. Two distinct impregnation processes were compared in this study.

The first impregnation process (designed as solid–liquid dispersion process) was an adaptation of the process used previously to impregnate ion-exchange microspheres (Chretien et al., 2004). Briefly, 0.5 g of freeze-dried microspheres was dispersed in 3 mL of a FITC-dextran-Manugel DMB sodium alginate solution, allowing its diffusion into the microspheres for 24 h at 25 °C. After removal of the excess sodium alginate solution by filtration through a 40-μm mesh, the microspheres-sodium alginate-dextran slurry was dispersed in 190 ml of deionised water under strong agitation (500 rpm) using a deflocculating propeller turbine. Ten seconds after the beginning of the agitation, the obtained microparticles were gelled by addition of a concentrated calcium chloride (10 mL, 2 M) solution to the dispersion for 1 h.

In the solid–solid dispersion process, the microspheres-sodium alginate-dextran slurry, prepared with Manugel DMB or with Keltone HVCR, was dried to constant weight in an oven at 25 °C. Microparticles were obtained by grinding of the obtained solid composite matrix in a mortar. Approximately 0.25 g of these microparticles was dispersed in 200 mL of a 0.1 M $CaCl_2$ gelation solution for 1 h or 48 h.

Finally, the gelled microparticles were rinsed with deionised water by filtration through a 40 μm mesh. The remaining microparticles were recovered and stored in 10 mL of deionised water.

The amount of FITC-dextran encapsulated was determined from 100 mg of rinsed water-swollen gelled microparticles after 24 h in 500 mL of pH 7.4 phosphate buffered saline (PBS). The amount of FITC-dextran released was analysed in the supernatant with a microplate fluorescent reader (FLx-800, BIOTEK Instruments) at excitation and emission wavelengths of 490 and 520 nm, respectively. The FITC-dextran encapsulation efficiency of the gelled microparticles was calculated as the ratio between the amount of FITC-dextran encapsulated and the amount of FITC-dextran dissolved in the sodium alginate solution. All studies were performed in triplicate and results were compared using one-way ANOVA tests.

2.3. Microparticle characterization

The particle size distribution of the gelled particles was analysed by laser light scattering (Malvern® Mastersizer, France) by the dispersion of 0.5 g of water-swollen microparticles in 50 mL of deionised water for 10 min before analysis. The particle size distribution data is presented as the relative frequency of the diameter based on the volume distribution of the microparticles. Samples were also observed by light microscopy to study the size and the shape of the microspheres using a Labophot-2A microscope (Nikon, France).

2.4. FITC-dextran release from impregnated microspheres

The influence of ionic strength on FITC-dextran release was tested as follows. Water-swollen microparticles (0.15 g) were dispersed in 500 mL of deionised water under a 100 rpm agitation. After 90 min, the microparticles were recovered by filtration and dispersed in 500 mL of PBS for 240 min. To study the influence of PBS volume, 0.15 g of filtered water-swollen microparticles was dispersed in 20 mL of PBS at 37 °C under a 100 rpm agitation. After 240 min, 480 mL of PBS was added for further 240 min. In both studies, the agitation was stopped 30 s before each sample collection to allow the microparticles to settle. A volume of 250 μL of the supernatant was sampled and frozen (−20 °C) until analysis.

The flow-through release studies were performed using USP type 4 dissolution method with 12 mm (internal diameter) cells. The conical bottom part of the cell was separated from the cylindrical portion by a 40 μm mesh nylon membrane (NY40, Millipore) between two #40 mesh screens. The filter head on top of the cell held a nylon filter mounted on a #40 mesh screen. Filtered water-swollen microparticles (0.15 g) were loaded in the cylindrical part of the dissolution cell. The release media were maintained at 37 ± 1 °C, and driven through the cell at a flow rate of 1 ml min^{-1} with a peristaltic pump. For each sample, deionised water was supplied to the flow-through cell for 90 min and PBS was used for the following 60 min. The eluent was sampled in a fraction collector (FRAC-200, Pharmacia) and subsequently frozen (−20 °C) until further required. FITC-dextran content in samples was measured spectrofluorimetrically.

The release data was fitted using an equation describing the mass transfer of a solute through a spherical particle into a perfect sink (Guy et al., 1982):

$$F = 6\sqrt{\frac{Dt}{\pi r_0^2}} \quad (1)$$

with F the fraction of dextran released, D its diffusion coefficient inside the microparticles and t the time. r_0, the microparticle radius, was assessed from the particle size distribution studies. For microparticles displaying highly variable radii, extreme values of r_0 were used to determine an approximate value for the diffusion coefficient. All studies were performed in triplicate and results were compared using one-way ANOVA tests.

3. Results and discussion

3.1. Characterization of the impregnated microspheres

In our previous paper (Chretien et al., 2004), the impregnated GF2000-Trisacryl microspheres were prepared using the solid–liquid dispersion process. When low sodium alginate concentrations (0.05 and 0.5%, w/v) were used, the preparation process yielded mononuclear impregnated microspheres. The impregnation of the microspheres with a 2% (w/v) sodium alginate solution induced their aggregation. This result was attributed to the increased viscosity of the alginate solution, which limited the dispersion of the microspheres in the high shear conditions induced by a deflocculating propeller turbine. In this study, the solid–liquid process was used to impregnate microspheres with 1–4% (w/v) sodium alginate solutions. With a 1% (w/v) sodium alginate solution, mononuclear microspheres were obtained. The increase in sodium alginate concentration lead to massive agglomerates (data not shown), from which only a small fraction of dispersed microspheres (inferior to 1% of the total gelled mass) could be recovered for further analyses.

To eliminate the influence of viscosity on the agglomeration of microspheres, a solid state dispersion process had to be adapted. The microspheres-sodium alginate-dextran slurry was dried, thereby forming a solid composite matrix. As no alteration of the microspheres had been observed after grinding of the dried GF2000-Trisacryl microspheres, the solid matrix was ground to prepare dextran-alginate-microparticles, which were then gelled in a calcium chloride solution. This alternative impregnation process allowed the formation of microparticles with sodium alginate solution concentrations ranging from 1–4% (w/v). For microparticles obtained from 1% (w/v) sodium alginate solution-impregnated microspheres, the study of particle size distribution (Fig. 1) displayed a fine particle size distribution, similar to the particle size distribution displayed by GF2000-Trisacryl microspheres. The particle size distribution of microparticles impregnated with 2% (w/v) sodium alginate solution figured a little

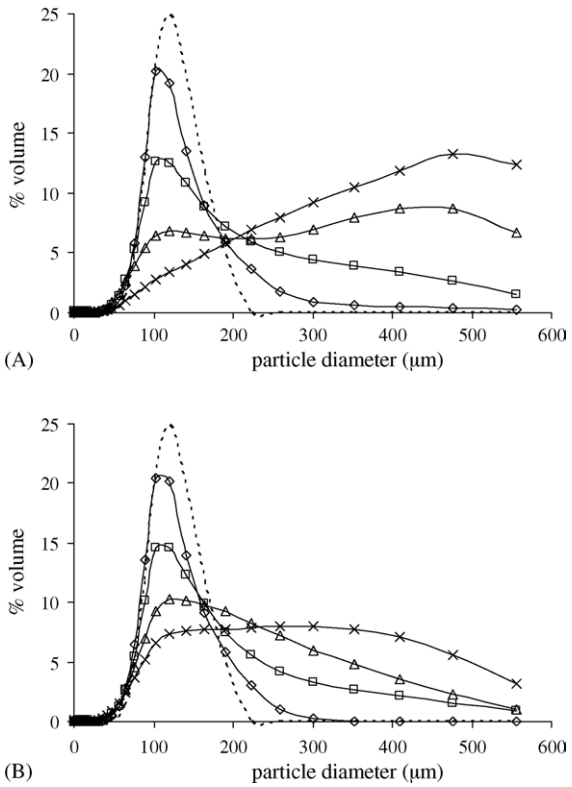

Fig. 1. Particle size distribution of microspheres impregnated using the solid–solid dispersion process. (A) Impregnation with Manugel DMB sodium alginate. (B) Impregnation with Keltone HVCR sodium alginate. Discontinuous line: GF2000-Trisacryl. Sodium alginate concentrations: (◊) 1%, w/v; (□) 2%, w/v; (△) 3%, w/v; (×) 4%, w/v. Gelation time had no influence on the particle size distribution; the plots obtained for the microspheres gelled for 48 h were not represented.

broader Gaussian curve. Observation of the obtained microparticles by light microscopy (data not shown) indicated that aggregation of the microspheres was limited. Microspheres impregnated with a sodium alginate concentration superior to 2% (w/v) figured a flattened and large Gaussian distribution with different diameters ranging from 100 to 600 nm. This result was attributed to the formation of polynucleated microparticles, with higher diameters than the microspheres, are generated. More microparticles with high diameters were observed for Manugel DMB-impregnated microspheres than for microspheres impregnated with Keltone HVCR.

These microparticles are composed of microspheres agglomerated with sodium alginate and FITC-dextran. The chemical structure of GF2000-Trisacryl microspheres, developed for the separation of biomacromolecules, implies the absence of ionic interactions between microspheres and sodium alginate. Thus, neither interactions between the microspheres and alginate, nor alginate viscosity would explain the agglomeration of the microspheres, as the dispersion step was performed in the solid state. The physical entanglement of alginate and microspheres would be a suitable explanation. The composition of the solid matrices, consisting of dried microspheres-sodium alginate-dextran slurries, suggests its heterogeneous hardness. Assuming a lower hardness of the sodium alginate, grinding of the solid matrices would have formed microparticles with a size distribution comparable to the GF2000-Trisacryl microspheres. This hypothesis fits with our results obtained for microspheres impregnated with 1 and 2% (w/v) sodium alginate solutions. According to Radebaugh (1990) and Li et al. (2002) regarding other polymers, the agglomerates formed with 3–4% (w/v) sodium alginate solutions are explained by the increase in the hardness of the dried form sodium alginate, figuring an homogenous the hardness of the solid composite matrix, thus generating higher microparticles diameters.

3.2. FITC-dextran encapsulation

According to Tanaka et al. (1984), the encapsulation of solutes in a calcium alginate gel is obtained for solutes with hydrodynamic radii superior to the hydrated pore radius of the gel. Several groups have described the encapsulation of 70 kDa—FITC-dextran by a one step-preparation process, namely droplet extrusion of a FITC-dextran-containing sodium alginate solution into a calcium chloride gelation solution (Kikuchi et al., 1997; Sezer and Akbuga, 1999). Attractive FITC-dextran encapsulation efficiencies were displayed (40–70%) (Sezer and Akbuga, 1999). In comparison to these studies, a preliminary dispersion step was necessary for the encapsulation of FITC-dextran in impregnated microspheres using the solid–liquid dispersion process: the microspheres-sodium alginate-FITC-dextran slurry was dispersed in deionised water for 10 s with a strong agitation. Then, encapsulation of FITC-dextran was obtained through the gelation of alginate by addition of a concentrated calcium chloride solution. The FITC-dextran encapsulation efficiencies obtained after the solid–liquid dispersion process ranged between 0.4 and 0.6% (data not shown), highlighting the dramatic loss of FITC-dextran during dispersion and gelation. The important loss of FITC-dextran is likely due to the dispersion step, as strong agitation and the physical state of sodium alginate facilitate the diffusion of FITC-dextran out of the microparticles towards the dispersion medium.

The FITC-dextran encapsulation efficiencies of the microspheres prepared using the solid–solid dispersion process ranged between 10 and 50% (Fig. 2). First, this result confirms the assumption formulated in the previous paragraph, highlighting the fast loss of FITC-dextran during the dispersion in deionised water. Therefore, the FITC-dextran encapsulation efficiencies determined from the microspheres prepared with the solid–solid dispersion process feature a major improvement of the preparation process. Nevertheless, the values obtained suggest FITC-dextran diffuses out of the microparticles during the gelation step, either before the attainment of equilibrium gelation or through diffusion in the gelled microparticle. Two different gelation times were tested to confirm this hypothesis. Results presented in Fig. 2A and B confirmed both hypotheses as the increase of the encapsulation efficiencies with reduction of the gelation time for each alginate concentration.

FITC-dextran encapsulation efficiencies are increased in microspheres impregnated with increased sodium alginate concentrations and gelled for 1 h (Fig. 2A and B). After a 48 h gelation of the microparticles, this trend was not reproduced and no significant difference was observed between various sodium

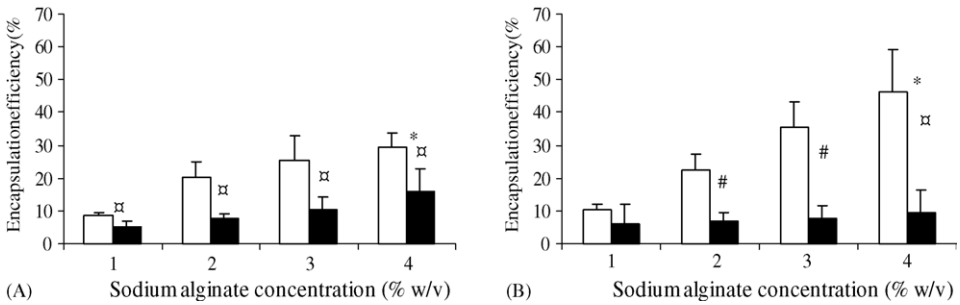

Fig. 2. Influence of alginate gelation conditions on FITC-dextran encapsulation efficiency. (A) Keltone HVCR; and (B) Manugel DMB. Gelation time: empty bars: 1 h; closed bars: 48 h. Statistical analysis (ANOVA): (*) difference induced by the increase in sodium alginate concentration ($p < 0.05$); (#) difference between 1 and 48 h gelation time ($p < 0.01$); (¤) difference between 1 h and 48 h gelation time ($p < 0.05$).

alginate concentrations. These results illustrate the loss of FITC-dextran by diffusion through the gelled microparticles, driven towards the gelation medium by the FITC-dextran concentration gradient. Equilibrium of the FITC-dextran concentration between the microparticles and the gelation medium was attained after 48 h. As the loss of FITC-dextran is driven by the concentration gradient, gelation of more microparticles in a smaller volume would increase the FITC-dextran encapsulation efficiency.

The highest encapsulation efficiencies have been obtained from microspheres impregnated with the Manugel DMB sodium alginate (Fig. 2B), which displays a lower M/G ratio than the Keltone HVCR alginate. The decrease in M/G ratio was reported to increase the gelation rate of alginate (Imai et al., 2000), and to decrease the diffusivity of solutes in calcium alginate (Amsden and Turner, 1999).

It should be noted that the presence of agglomerates in some of our preparations should be considered as another explanation for our results. Upon the increase of the concentration of sodium alginate solution, more agglomerates are formed. In these microparticles, the diffusion path of FITC-dextran is increased, and the loss of FITC-dextran is therefore limited after 1 h-gelation. The results we obtained are likely dependent on both agglomerates and the composition of our microparticles in terms of alginate composition, and concentration of the sodium alginate.

3.3. Release conditions

The first step of our release studies was to study the influence of the release medium composition on FITC-dextran release in order to study the influence of the reversible ionic interactions between the carboxylate moieties of alginate and calcium ions on the encapsulation of FITC-dextran. Dispersed in deionised water as a release medium, a small fraction of the encapsulated FITC-dextran was released (Fig. 3). The release equation developed by Guy et al. (1982) seemed appropriate to fit the release data. The best results were obtained for a diffusion coefficient of FITC-dextran (70 kDa) in the microparticle of 10^{-16} to 10^{-15} $cm^2\,s^{-1}$. As the diffusion coefficients of a low molecular weight drug, indomethacin, in the impregnated microspheres were of approximately 10^{-6} $cm^2\,s^{-1}$ (Chretien et al., 2004), the slower diffusion of FITC-dextran highlights the influence of molecular weight of solutes on their diffusion through porous hydrogels (Amsden, 1998).

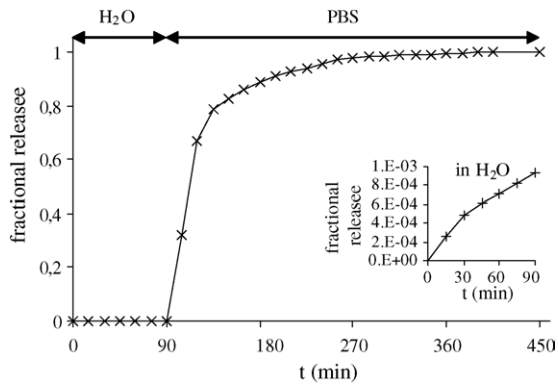

Fig. 3. Influence of deionised water and PBS on FITC-dextran release from 4% (w/v) Manugel DMB-impregnated microspheres in 500 mL release media. The inserted figure represents an enlargement of the FITC-dextran release in deionised water.

In PBS, the full FITC-dextran payload was quickly released (Fig. 3). This burst effect suggests a modification in the impregnated microspheres. Wang and Garth (1998) have described the swelling of gelled alginate beads exposed to inert electrolytes such as potassium chloride. The exchange of the divalent calcium involved in electrostatic links between various carboxylate moieties of the alginate chain with the monovalent sodium leads to an increased osmotic pressure inside the gel, causing it to swell. In our study, the high concentration of sodium ions in PBS and the presence of phosphate ions able to interact with calcium ions to form insoluble complexes induced the exchange of calcium with sodium ions. The swelling of the alginate impregnated in the microspheres increased their porosity, thereby allowing the quick release of FITC-dextran from the microparticles. The diffusion coefficient of FITC-dextran (10^{-9} to 10^{-8} $cm^2\,s^{-1}$) in the swollen alginate was still inferior to the values obtained for indomethacin (Chretien et al., 2004).

The second step of the study of release conditions considered the influence of monovalent cations quantity on FITC-dextran release. Although a decrease in the ionic force of the release medium would have allowed a physicochemical study of the influence of the monovalent cations quantity on FITC-dextran release, we focussed on the influence of volume, featuring a release medium with an ionic force similar to physiological conditions (0.165 M). Fractional release of FITC-dextran in 20 mL and in 500 mL of PBS is presented in Fig. 4. A smaller fraction of FITC-dextran was released in the 20 mL volume than in the 500 mL volume. Furthermore, the release medium volume had an influence on the release kinetics (10^{-13} to 10^{-11} $cm^2\,s^{-1}$ in the smaller volume and 10^{-9} to 10^{-8} $cm^2\,s^{-1}$ in the 500 mL volume). The diffusion coefficient of FITC-dextran in the 20 mL volume suggests that diffusion in PBS was facilitated compared to diffusion in deionised water. Thus, the amount of sodium ions induced the swelling of the gelled alginate impregnated in the microspheres.

To analyse this result, it should be pointed out that in 20 mL of PBS, the release of FITC-dextran is performed in a perfect sink. Thus, its solubility does not interfere with its release kinetic. From this point, the comparison between the FITC-dextran release data in the 20 mL volume and in the 500 mL volume raises two hypotheses. The lower extent released in the smaller

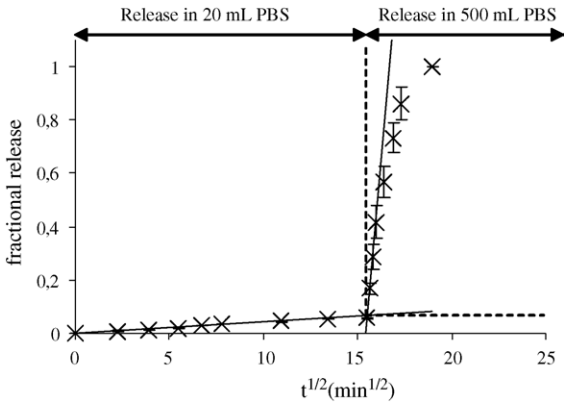

Fig. 4. Influence of PBS volume on FITC-dextran release kinetics presented as the release data plotted against the square root of time for 4% (w/v) Manugel DMB- impregnated microspheres. Discontinuous lines represent the alternative referential in 500 mL PBS. Continuous lines depict the linear regression between fractional release and square root of time. The diffusion coefficients calculated from the slopes were 10^{-13} to 10^{-11} $cm^2\,s^{-1}$ ($R^2 = 0.993$) and 10^{-9} to 10^{-8} $cm^2\,s^{-1}$ ($R^2 = 0.999$) in 20 mL and in 500 mL, respectively.

release volume is likely due to the fact that diffusion is driven by the concentration gradient. In the 20 mL volume, FITC-dextran in the release medium will reach higher concentrations than in the 500 mL volume, thus depressing the concentration gradient, thereby leading to a slower release. The second hypothesis considers the ratio between the concentrations of sodium ions and calcium ions. Martinsen's group defined the minimal calcium to sodium molar ratio to provide calcium alginate gel swelling and erosion (Martinsen et al., 1989), depending on the composition of alginate. In the smaller volume, the extent of the exchange between sodium and calcium ions features an alternate situation with a limited swelling, thus leading to the partial release of FITC-dextran. In the 500 mL volume, the quantity of sodium ions allows the complete swelling of the alginate impregnated in the microspheres, allowing the release of the full FITC-dextran payload.

3.4. Release in a continuous flow

There are no guidelines for in vitro release studies of parenteral drug delivery systems (Burgess et al., 2002). Release studies were performed using PBS as a release medium, as its ionic strength (0.165 M) is similar to the ionic strength of plasma. To fit with the characteristics

of arterial blood (flow rate and volume in contact with the microspheres per unit time), our microspheres were studied in the USP type 4 dissolution apparatus, which displays a limited volume of about 10 mL in contact with the microspheres per unit time. An infinite release medium volume was flushed in a laminar flow through the apparatus.

First, we observed the linear release of FITC-dextran in deionised water (1×10^{-6} to 1×10^{-5} mg min^{-1}). A 90 min study did not show any release rate reduction. Continuous refreshment of the release medium lead to the absence of saturation, in contrast to release in a finite release volume. The comparison between the various formulations did not show any difference in the release rates. Thus, the diffusion rate of FITC-dextran through the gelled microspheres was independent of the fixed characteristics of the formulations (sodium alginate concentration and sodium alginate M/G ratio) in our in vitro release conditions in the flow-through apparatus. Furthermore, their diffusion rates were independent of the particle size distribution of the gelled microspheres.

The release in PBS allowed the study of the release mechanisms in a reduced volume of PBS with the constant renewal of the release medium. Fig. 5 represents the release profiles of various microparticle formulations. For most of them, the release profiles were sigmoid, whereas the release profiles in a limited volume presented above could be linearized when plotted against the square root of time. Release profiles obtained in the flow-through apparatus consist of two subsequent phases. The first phase resembles the release in the 20 mL volume and represents the slow release of FITC-dextran. The second phase features an increase in the release rate leading to the release of the full payload of FITC-dextran. The concentration

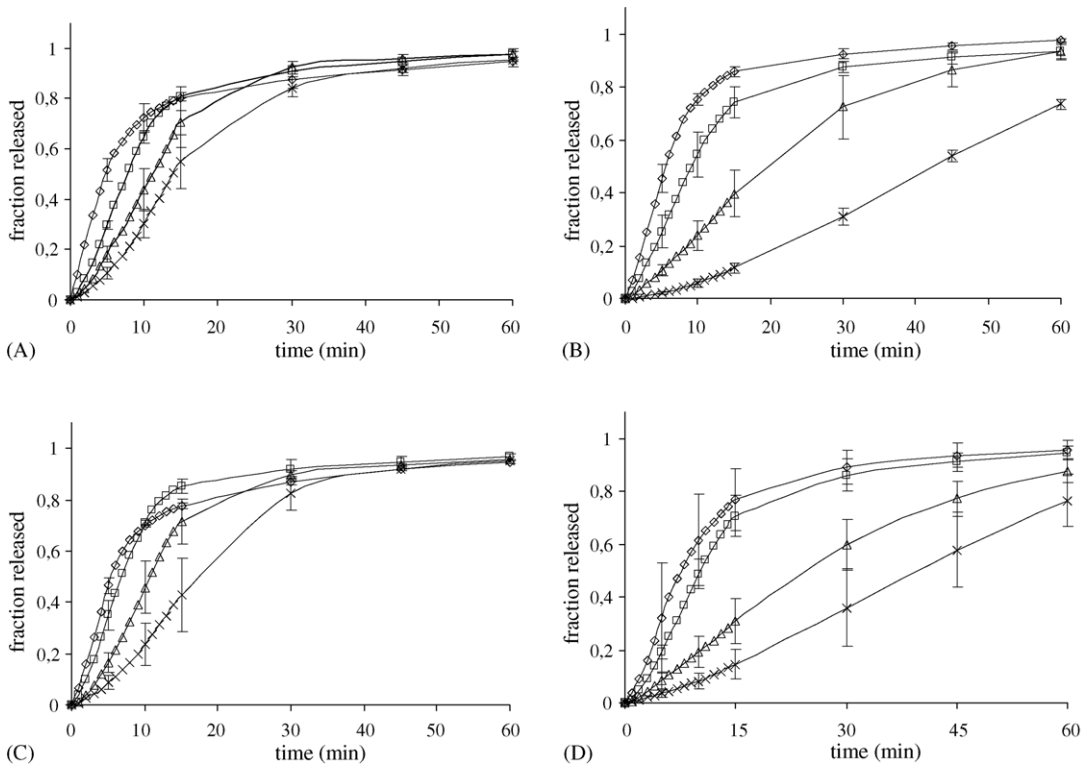

Fig. 5. Cumulated FITC-dextran release in the flow-through apparatus. (A) Impregnation with Keltone HVCR sodium alginate (gelled for 1 h). (B) Impregnation with Manugel DMB sodium alginate (gelled for 1 h). (C) Impregnation with Keltone HVCR sodium alginate (gelled for 48 h). (D) Impregnation with Manugel DMB sodium alginate (gelled for 48 h). Sodium alginate concentrations: (◊) 1%, w/v; (□) 2%, w/v; (△) 3%, w/v; (×) 4%, w/v.

gradient-limited release hypothesis fails to explain the existence of these two phases. On the other hand, the constant renewal of the release medium would progressively increase the molar ratio between sodium ions and calcium ions involved in electrostatic links with the alginate impregnated in the microspheres, thereby progressively increasing the swelling degree of alginate and facilitating the diffusion of FITC-dextran out of the microparticles. Kikuchi's group (Kikuchi et al., 1997) reported the bimodal erosion of calcium gels in a sodium ions-containing medium. First, the disruption of the interactions between calcium and mannuronates units lead to the swelling of the gel. The second phase appeared after a lag time and represented the disruption of the interactions between polyguluronates units and calcium. Furthermore, Wang and Garth (1998) calculated the respective affinities of mannuronate units and guluronate units for calcium ions. According to these studies, the progressive swelling of the alginate impregnated in the microspheres is explained by the disruption of calcium-mannuronate interactions. The increase in osmotic force in the partly eroded gel and the constant renewal of the release medium lead to the complete erosion of the alginate-calcium, inducing the second phase of the release. Nevertheless, it should be noted that the sigmoid release is a consequence of the flow rate used in this study. Its increase would probably lead to release profiles similar to those observed in a 500 mL batch volume of PBS. Therefore, in vivo release studies are necessary to confirm the sigmoid shape of the release profile of our impregnated microspheres.

For the different formulations, the first phase of the release lasted from a few seconds to more than 5 min depending on alginate concentration and on alginate type. Furthermore, a reduced release rate was observed for 3 and 4% (w/v) sodium alginate-impregnated microspheres. To compare the release profiles of our microspheres, we plotted our release data against the square root of time (Fig. 6). Interestingly, only the data from the second phase fitted a linear trend, suggesting the release of dextran is controlled by diffusion in the eroded microparticle (Higuchi, 1963). Moreover, parallel slopes were obtained from formulations prepared with various sodium alginate concentrations. We assumed that parallel slopes were the result of the diffusion in eroded microparticles having the same porosity and the same diffusion path (Higuchi, 1963). Therefore, release slopes were chosen as an assessment of the particle diameter. Earlier, we observed a dramatic influence of sodium alginate concentration and sodium alginate M/G ratio on particle size distribution. A systematic statistical comparison of the slopes of the release data was performed using ANOVA-tests. We stated particle size distribution had a minor influence on release profiles for formulations displaying similar release slopes. Furthermore, the intensity of the shifts parallel to the abscissa of similar release slopes is a convenient method to determine the time length of the first phase of the release. Slopes and shifts values are presented in Table 1.

Fig. 6A and C show that the increase in Keltone HVCR concentration increased the time length of the first phase of the release profile as the release slopes representing the second phase were parallel. The same observation was made for 1% (w/v)- and 2% (w/v)-Manugel DMB-impregnated microspheres (Fig. 6B). This trend suggests that the increase in alginate concentration increases the time for attainment of equilibrium swelling. Thus, the time for attainment of equilibrium is controlled by the sodium ions renewal rate, i.e. the flow rate of the release medium. The influence of gelation time or alginate composition did not show any influence on the first phase of the release.

Table 1
Characteristic values describing the release profiles

Alginate type	Gelation time (h)	[Na-Alg]	Slope ($min^{-1/2}$)	Shift (min)
Keltone HVCR	1	1% (w/v)	0.32 ± 0.08	0.55 ± 0.48
		2% (w/v)	0.36 ± 0.01	1.87 ± 0.08
		3% (w/v)	0.34 ± 0.05	3.36 ± 0.20
		4% (w/v)	0.30 ± 0.07	4.27 ± 0.39
Manugel DMB	1	1% (w/v)	0.33 ± 0.08	0.88 ± 0.39
		2% (w/v)	0.28 ± 0.02	1.42 ± 0.49
		3% (w/v)	0.21 ± 0.05	3.31 ± 0.08
		4% (w/v)	0.10 ± 0.07	4.09 ± 1.03
Keltone HVCR	48	1% (w/v)	0.36 ± 0.01	0.85 ± 0.15
		2% (w/v)	0.34 ± 0.04	1.61 ± 0.34
		3% (w/v)	0.39 ± 0.03	3.91 ± 0.33
		4% (w/v)	0.25 ± 0.06	4.57 ± 0.01
Manugel DMB	48	1% (w/v)	0.30 ± 0.02	1.49 ± 1.61
		2% (w/v)	0.31 ± 0.04	2.59 ± 0.25
		3% (w/v)	0.17 ± 0.02	4.41 ± 1.85
		4% (w/v)	0.16 ± 0.01	9.33 ± 5.11

[Na-Alg]: sodium alginate concentration.

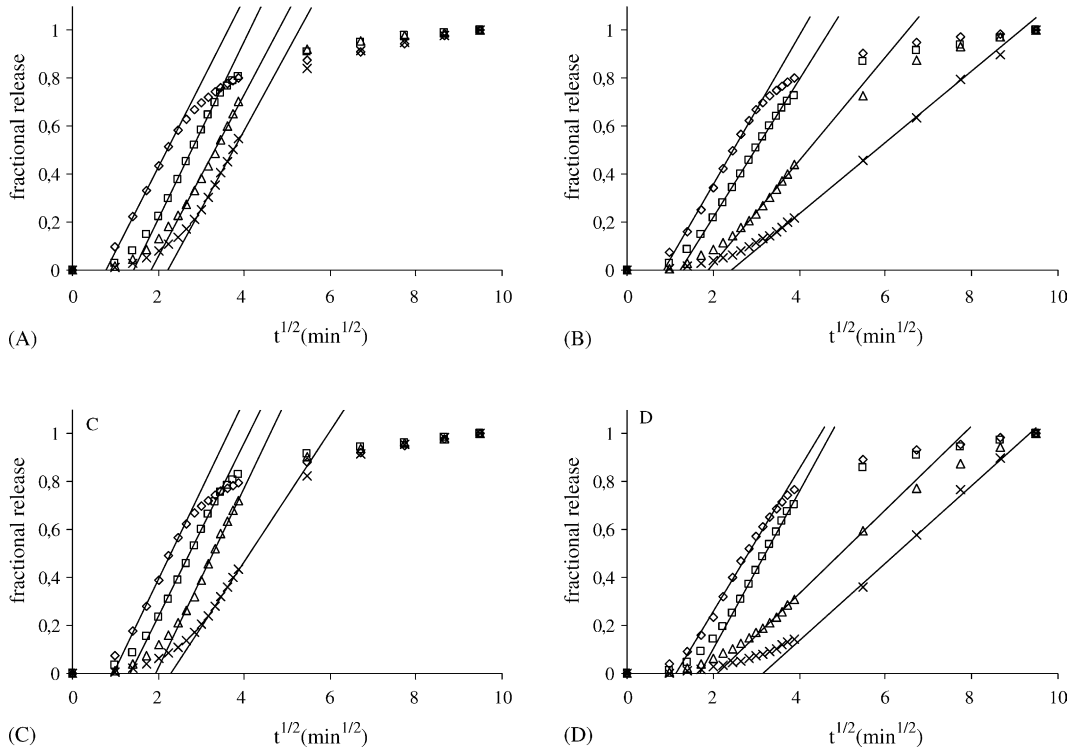

Fig. 6. Extraction of linear sections from FITC-dextran release data plotted against the square root of time. (A) Impregnation with Keltone HVCR sodium alginate (gelled for 1 h). (B) Impregnation with Manugel DMB sodium alginate (gelled for 1 h). (C) Impregnation with Keltone HVCR sodium alginate (gelled for 48 h). (D) Impregnation with Manugel DMB sodium alginate (gelled for 48 h). Sodium alginate concentrations: (◊) 1%, w/v; (□) 2%, w/v; (△) 3%, w/v; (×) 4%, w/v.

4. Conclusion

This work presents a technological platform for high molecular weight drugs. Impregnation of macroporous microspheres with calcium alginate allowed the encapsulation of FITC-dextran. Calcium ions exchange with sodium ions triggered its fast release by erosion of impregnated calcium alginate after a controlled time, which depended on the concentration of the alginate impregnated in the microspheres. Further confirmations of our results will be obtained by FITC-dextran release studies during embolization studies.

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