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Preparation and characterization of cisplatin-loaded polymethyl methacrylate microspheres

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Summary

Non-biodegradable microspheres containing cisplatin were prepared by the solvent evaporation method, to achieve a sustained drug delivery system for local chemotherapy in bone tumors. Polymethyl methacrylate (PMMA) was chosen as coating material, since this polymer is widely used as a bone cement in orthopaedic surgery, particularly in total hip arthroplasty. The present study was carried out to show how various process parameters could influence the preparation of PMMA microspheres and their properties. Microsphere size and morphology were controlled by the nature and the concentration of the emulsifying agent. The amount of cisplatin that could be incorporated into the microspheres depended on the solubility of the drug in the aqueous phase and the rate of precipitation of PMMA at the droplet surface. The drug content was also influenced by the organic solvent : aqueous phase ratio. In vitro drug release was increased by addition of a porosity agent. The different parameters were optimized in order to achieve various drug release rates and high cisplatin loadings. Successful results were obtained with cisplatin, a highly water-soluble compound, whereas the solvent evaporation method is usually used for the entrapment of lipophilic drugs.

Introduction

The use of specific polymers and the design of new drug delivery dosage forms are currently perhaps one of the most exciting areas in pharmaceutical formulation. The major aim is to improve the efficiency of the treatment and to decrease its side effects. The use of biomaterials able to deliver the drug to its specific target organ can represent an interesting approach.

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We especially investigated polymethyl methacrylate (PMMA) as coating material for a sustained release drug delivery device. One of the first applications of this biomaterial in orthopaedic surgery was its use as bone cement in association with antibiotics to control infection in total hip arthroplasty (Buchholz et al., 1970; Marks et al., 1976; Röttger et al., 1979).

Another application field could be represented by the treatment of osteosarcoma. The parenteral administration of antimitotic drugs is widely used for primary bone tumors such as osteosarcoma and sometimes for secondary tumors. But even parenteral chemotherapy and wide surgical exci-

sion do not always prevent local recurrence or metastasis from bone sarcomas. For these reasons, it was thought that the efficiency of treatment might be increased by local chemotherapy from antimitotic drug-loaded microparticles. The eradication of the sensitive tumor cells will therefore be facilitated by exposing them to high concentrations of anticancer drug. Recently, the possible use of acrylic cement containing a chemotherapeutic drug was investigated in the treatment of malignant lesions in bone (Hernigou et al., 1987; Hernigou et al., 1989).

The specific aims of this study were to prepare PMMA microspheres containing cisplatin, a cytostatic agent whose effectiveness on bone tumors is well known, to optimize the different manufacturing process parameters and to characterize the in vitro dissolution kinetics of cisplatin from microspheres.

Materials and Methods

Materials

Cisplatin bulk powder was obtained from Laboratoires Roger Bellon (Neuilly-sur-Seine, France). Polymethyl methacrylate was provided in bone cement Cerafix® (Ceraver, Courbevoie, France) as coating polymer. Polyvinyl alcohol (PVA) 4/125 (Rhodoviol®) was supplied by Prolabo (Paris, France). The different grades of methyl celluloses employed were Methocel® 10 and 400 mPa s (Dow Chemical, Paris, France). Gelatin (200 Blooms) was provided by Rousselot (Paris, France). Polyoxyethylene glycol (PEG 4000), sodium chloride, sodium hydrogenosulfate (Na_2HPO_4), sodium dihydrogenosulfate (NaH_2PO_4), potassium hydrogenosulfate (K_2HPO_4) and potassium dihydrogenosulfate (KH_2PO_4) were provided by Prolabo (Paris, France). Methylene chloride and ethanol were used without further purification (Prolabo, Paris, France).

Preparation of microspheres

Microspheres were prepared by an oil-in-water (o/w) emulsion solvent evaporation method, which was adapted from the process described by Beck et al. (1979). PMMA (1 g) was dissolved in organic solvent. Cisplatin was dispersed into this solution, in various amounts corresponding to different drug loadings. This polymeric phase was then added to 250 ml of an aqueous solution containing an emulsion stabilizer. Agitation was maintained at 300 rpm until complete evaporation of the organic solvent. Microspheres were then collected, washed three times with deionized water, filtered and stored under reduced pressure overnight in a desiccator.

Microscopic examination and size determination

Particle size was determined using a projection microscope (Olympus, Tokyo, Japan). At least 200 microspheres were dispersed on a slide and their diameter was then sized using suitable objectives.

Determination of drug content in microspheres

A weighed quantity of microspheres was dissolved in dimethylformamide, which is a good solvent for both PMMA and cisplatin. Platinum was assayed spectrophotometrically at 402 nm (Perkin Elmer, Bois d'Arcy, France) after complexation with stannous chloride, and cisplatin loading was then calculated.

The encapsulation efficiency was determined from the ratio of measured to theoretical cisplatin loading.

In vitro release studies

In vitro release studies of cisplatin from microspheres were carried out in round-bottomed flasks under sink conditions. A weighed quantity of microspheres was suspended in normal saline adjusted to pH 4 with HCl (1 N). The dissolution medium was stirred at 100 rpm and maintained at constant temperature (37°C) in a water bath. Aliquots were withdrawn at specified time intervals for a duration of 21 days; to maintain a constant volume, a volume of dissolution medium equal to that of the aliquot was immediately added after each sample was removed. Platinum was assayed spectrophotometrically at 402 nm after complexation with stannous chloride.

The objectives of the current investigations

were to optimize the particle preparation process in order to achieve high cisplatin loadings, narrow size distribution and good drug release properties. In this study, the effects of the following preparation variables were investigated:

(i) Nature and concentration of emulsion stabilizer. The effect of different tensio-active agents (PVA, Methocel 10, Methocel 400, gelatin 200) on microsphere size distribution was studied.

(ii) Composition of the aqueous phase. The addition of different salts (NaCl, Na_2HPO_4, NaH_2PO_4, K_2HPO_4 and KH_2PO_4) to the aqueous medium was performed to examine the influence on microsphere morphology and cisplatin loading.

(iii) Nature of the organic phase. This was studied by comparing methylene chloride and methylene chloride/ethanol mixtures on cisplatin encapsulation.

(iv) Concentration of the polymer solution. This was studied by variation of the organic solvent volume in which a fixed weight of polymer was dissolved (1 g PMMA dissolved in 20, 10, 8 and 5 ml organic phase). The optimum concentration was determined based on the cisplatin encapsulation efficiency and microsphere size distribution.

(v) Addition of a porosity agent. PEG 4000 was added to the organic phase to modify the rate of release of cisplatin from microspheres. The influence of this porosity agent on cisplatin release was evaluated by studying the in vitro release characteristics of four different batches of microspheres prepared by varying the concentration of PEG 4000 from 0 to 30% of polymer weight.

Results and Discussion

Nature and concentration of emulsion stabilizer

The microsphere morphology and size distribution obtained with gelatin, methyl cellulose 400, methyl cellulose 10 (0.25% w/v) and PVA (0.25 and 1% w/v) are presented in Table 1.

The choice of surfactant significantly affected particle size and, for a 0.25% w/v surfactant concentration, gelatin 200 Blooms and methyl cellulose 400 mPa s produced a population of aggregated microspheres with a broad size distribution. These results could be explained by the high molecular weight of the stabilizer and accompanying high viscosity of the resulting aqueous phase, thus preventing the proper dispersion of the organic medium. With methyl cellulose 10 mPa s, the size range was slightly wider than with PVA and therefore PVA was selected for further studies as an emulsifier agent able to provide a narrow and satisfactory microsphere size distribution. An increase in PVA concentration (1% w/v) resulted in an increase in particle size. A possible explanation, in this case, could also be the effect of the high viscosity of the resulting aqueous phase, and a PVA concentration of 0.25% (w/v) was selected definitely for microsphere preparation.

Composition of the aqueous phase

Cisplatin is a rather water-soluble compound (1 g/l), while the solvent evaporation process is a method more suitable for microencapsulation of lipophilic drugs. Therefore, this method might be

TABLE 1

Effect of emulsifier agent on size distribution and morphology of microspheres

Emulsifier (concentration % w/v)	Size distribution (mean ± SD) (μm)	Microsphere morphology
Gelatin (0.25)	203 ± 57	spherical particles, aggregation
Methyl cellulose 400 (0.25)	117 ± 42	spherical particles, aggregation
Methyl cellulose 10 (0.25)	92 ± 20	spherical, individualized particles
PVA (0.25)	81 ± 15	spherical, individualized particles
PVA (1)	99 ± 20	spherical, individualized particles

optimized in order to reduce drug loss in the aqueous phase during the emulsifying step. Some authors have shown that the amount of drug incorporated into microspheres could be improved by prior saturation of the aqueous phase with the drug (Spenlehauer et al., 1986; Bodmeier and McGinity, 1987). Because cisplatin is expensive, another possible improvement of the encapsulation method was to decrease the hydrosolubility of the cisplatin by adding different salts to the aqueous medium until saturation. Additionally, it was necessary to employ optimal stability conditions for cisplatin: the presence of chloride ions and low pH to prevent the conversion of cisplatin into monoaquo and diaquo derivatives (Gouyette, 1984). The pH of the aqueous phase was therefore adjusted to 4 with concentrated HCl. The effects of the different salts on microspheres morphology are listed in Table 2. Only two salts provided regular and well individualized microspheres: NaH_2PO_4 and overhead KH_2PO_4. In terms of encapsulation efficiency, the influence of saturating the dispersing phase with this salt was evident, as shown in Table 3. For all further experiments, we therefore dissolved KH_2PO_4 in the aqueous phase until saturation.

Nature of the organic phase

Despite the addition of KH_2PO_4, drug partitioning into the aqueous phase still occurred during the initial stage of microsphere formation prior to polymer precipitation. Consequently, a cosolvent was added to methylene chloride, in order to increase the rate of polymer precipitation. Ethanol was chosen because of its high water solubility, promoting the precipitation of the polymer droplets by rapidly diffusing out of the globules into the aqueous phase. The use of a methylene chloride/ethanol ratio of 2:1 made it possible to encapsulate greater amounts of cisplatin. For a cisplatin/PMMA ratio of 0.1:1, the encapsulation efficiency increased to 57.4% with ethanol, in comparison to 31.6% with methylene chloride alone.

These results are in good accordance with previous studies, showing that the addition of water-miscible cosolvents such as acetone or methanol resulted in an increase in drug content as a result of the faster precipitation of the polymer (Bodmeier and McGinity, 1988).

TABLE 2

Influence of different salts as aqueous phase-saturating agents on microsphere morphology (all microspheres prepared with 0.25% w/v PVA)

Salt	Microsphere morphology
NaCl	aggregated microspheres
Na_2HPO_4	irregular particles
K_2HPO_4	irregular particles
NaH_2PO_4	spherical, individualized microspheres
KH_2PO_4	regular, spherical, individualized microspheres

TABLE 3

Effect of KH_2PO_4 as aqueous phase-saturating agent on cisplatin encapsulation efficiency (all microspheres prepared with 0.25% w/v PVA)

Cisplatin/PPMA ratio (g:g)	KH_2PO_4 (saturation)	Cisplatin loading (% w/v)	Encapsulation efficiency (%)
0.05:1	–	0.82	17.2
	+	1.61	33.8
0.1:1	–	1.52	16.7
	+	2.87	31.6
0.2:1	–	2.06	12.4
	+	4.08	24.5

Concentration of the polymer solution

In an attempt to identify the optimum conditions for the preparation of microspheres, the influence of polymer concentration in the organic phase was investigated, without changing the previous variables allowing the best encapsulation ratio. The results on encapsulation efficiency and size distribution are given in Table 4. Dissolving a fixed weight of polymer (1 g) in decreasing volumes of solvent resulted in an effective improvement in encapsulation efficiency. In the same way, the higher concentration of PMMA in the solvent caused the microsphere size distribution to shift towards higher particle size. It can be concluded from these results that increasing the polymer/solvent ratio produced a more viscous dispersion, which permitted more rapid precipita-

TABLE 4

Influence of polymer concentration in the organic phase (CH_2Cl_2 / ethanol 2:1) on encapsulation efficiency and microsphere size distribution (all microspheres prepared with 0.25% w/v PVA, KH_2PO_4 as saturating agent and drug / polymer ratio of 0.1:1)

PMMA/organic phase ratio (g:ml)	Encapsulation efficiency (%)	Size distribution (mean ± SD) (μm)
1:20	57.4	81 ± 15
1:10	72.7	93 ± 30
1:8	88.0	104 ± 38
1:5	90.1	254 ± 108

tion of polymer but also formation of larger droplets. 8 ml was selected as an appropriate volume of organic solvent for providing microspheres with a high encapsulation efficiency (88%) and the proper size distribution (104 ± 38 μm).

Addition of a porosity agent

As demonstrated in Table 5, different concentrations of PEG 4000 (10, 20 and 30% of polymer amount) did not significantly affect cisplatin loading for microspheres prepared with a drug/polymer ratio of 0.1:1. However, increasing the amount of PEG 4000 led to a shift in the microsphere size distribution towards higher particle size. This was due to the fact that a higher concentration of PEG produced a more viscous dispersion which formed larger droplets in the aqueous phase and consequently, larger microspheres.

Fig. 1 shows the profiles for cisplatin release from PMMA microspheres prepared with different amounts of PEG 4000. None of the samples released a major fraction of cisplatin immediately upon immersion in normal saline. The possible presence of a burst effect could be excluded. Additionally, the greater the amount of PEG 4000, the faster was the release rate. Similar results have been reported by others with indomethacin-loaded ethylcellulose microspheres (Babay et al., 1988) and with theophylline-loaded PMMA microspheres prepared by an emulsion non-solvent addition method (Pongpaibul et al., 1988).

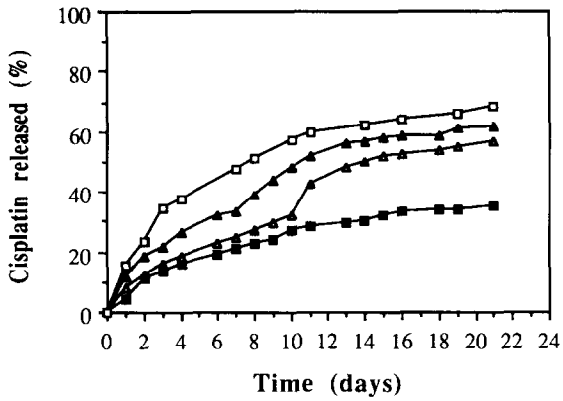

Fig. 1. Influence of PEG 4000 (% of polymer amount) on in vitro cisplatin release from microspheres (all microspheres prepared with 0.25% w/v PVA, KH_2PO_4 as saturating agent, PMMA to organic phase ratio of 1:8 and drug/polymer ratio of 0.1:1). PEG (■) 0%, (▲) 10%, (▲) 20%, (□) 30%.

TABLE 5

Effect of PEG 4000 on cisplatin encapsulation efficiency and microspheres size distribution (all microspheres prepared with 0.25% w/v PVA, KH_2PO_4 as saturating agent, PMMA / organic phase ratio of 1:8 and drug / polymer ratio of 0.1:1)

PEG 4000 amount (% polymer amount)	Encapsulation efficiency (%)	Size distribution (mean ± SD) (μm)
0	88.0	104 ± 38
10	84.2	111 ± 53
20	84.6	110 ± 49
30	89.1	131 ± 48

In view of these findings, it is believed that cisplatin cannot readily diffuse into the aqueous phase when there is no porosity agent in the coating polymer. In contrast, when PEG 4000 is incorporated into the polymer matrix, it could dissolve and simultaneously leave channels, promoting the diffusion of water into the microspheres and the dissolution of encapsulated cisplatin.

In conclusion, these investigations have provided an understanding of the effects of several process parameters on microsphere properties. We have achieved the successful entrapment of cisplatin into PMMA microspheres. Although the solvent evaporation method is usually applied for the encapsulation of lipophilic drugs, selection of the appropriate conditions has enabled the

preparation of spherical cisplatin-loaded PMMA microspheres with high drug encapsulation efficiency, narrow size distribution and various in vitro drug release rates.

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