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The preparation and characterisation of poly(lactide-co-glycolide) microparticles. I: Oil-in-water emulsion solvent evaporation

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Summary

An oil-in-water emulsion solvent evaporation technique has been used to prepare poly(lactide-co-glycolide) (PLGA) microparticles and the effect of various process parameters on particle size has been investigated. Particles of below 3 μm mean particle size were prepared by using a relatively small amount of polymer, a high stirring rate and a low volume of aqueous phase containing a high concentration of surfactant.

Introduction

Although effective vaccines are available for a number of diseases, many of the current vaccines have the disadvantage of requiring several doses in order to achieve protective immunity. This requires several contacts between the vaccine-recipient and health-care workers which causes problems particularly in Third World countries where often only a small proportion of individuals actually return for the booster doses of vaccines (Bloom, 1989). The development of a single-dose controlled release vaccine would eliminate the need for booster immunisations and would be a significant advance in the efforts to protect individuals against a number of vaccine preventable diseases.

Recently, poly(lactide-co-glycolide) (PLGA) microparticles have been investigated as antigen delivery systems for use as controlled release vaccines (O'Hagan et al., 1991). PLGA microparticles have proved to be successful drug delivery systems in many formulations involving a range of drugs, including peptides and proteins (Ogawa et al., 1988; Hora et al., 1990). PLGA is a biocompatible, biodegradable synthetic polymer which degrades at a rate dependent on properties such as polymer molecular weight, crystallinity and lactide:glycolide ratio (Cutright et al., 1974; Hutchinson and Furr, 1986). By selection of the appropriate polymer composition with a known rate of degradation, the polymers can be exploited to produce a drug delivery system which releases an active agent at a predetermined rate.

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A recent study in mice has indicated that antigens entrapped in microparticles are capable of inducing potent antibody responses following parenteral immunisation (O'Hagan et al., 1991). It was postulated that the potent immune responses were a result of more efficient antigen presentation due to phagocytosis of the microparticles by tissue macrophages. If this is so, then it is clear that particle size will have an important role to play in promoting the efficacy of microparticulate vaccines.

A prerequisite of an ideal vaccine is that it should be administered orally, since this route offers a number of advantages, including ease and convenience of administration, reduced cost and minimal side-effects. Microparticles have considerable potential as oral vaccines, since they are taken up across the gastrointestinal tract (GIT) following oral administration (O'Hagan, 1990). Indeed, in several recent studies, microparticles have been successfully used as oral antigen delivery systems for entrapped antigens (O'Hagan et al., 1989; Eldridge et al., 1990).

Although the factors controlling particle uptake across the GIT are poorly defined, particle size has been shown to be an important parameter. In one study in mice, PLGA microparticles as large as 10 μm were apparently taken up (Eldridge et al., 1990). However, in an alternative study in rats by Jani et al. (1989), polystyrene

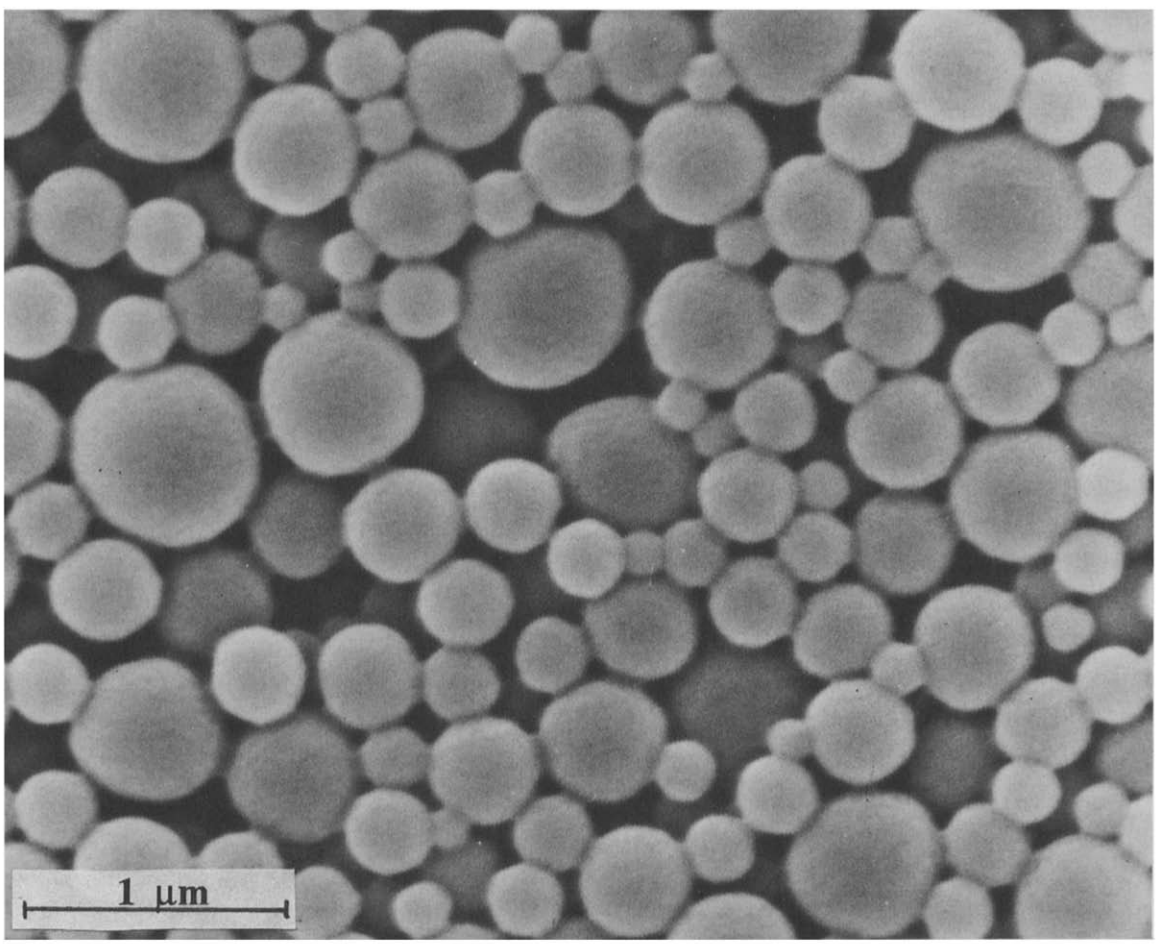

Fig. 1. PLGA microparticles of average size 0.9 μm as shown under scanning electron microscopy. Magnification $\times 35\,000$.

particles less than 1 μm in size showed optimal uptake and particles of 3 μm were apparently not taken up.

If the potential of microparticulate controlled release vaccines is to be realised, then the control of particle size is essential. Consequently, we have adapted a technique first described by Beck et al. (1979) to produce relatively large microparticles (43–61 μm), to produce PLGA microparticles below 3 μm in diameter (Fig. 1). This paper describes the effect of various process parameters on particle size and defines the preparation conditions necessary for the production of particles below 3 μm in size.

Materials and Methods

Materials

Poly(d,l-lactide-co-glycolide): co-polymer compositions, 50:50 (molecular mass 9 kDa); 50:50 (22 kDa); 75:25 (18 kDa) and 85:15 (53 kDa) were donated by Alpha Chemicals, Preston. Polyvinyl alcohol (13–23 kDa, 87–89% hydrolysed) and methylcellulose (400 cps) were supplied by Aldrich Chemical Co., Dorset. Tween 80, gelatin (175 Bloom Type A) and poloxamine 908 were obtained from Sigma Chemical Co., Dorset. Sodium dodecyl sulphate was supplied by BDH, cetyltrimethyl ammonium bromide by Fluka AG, Buchs, Germany and dichloromethane (HPLC grade) by May and Baker, Essex. All materials were used as supplied.

Preparation of microparticles

An oil-in-water (o/w) emulsion solvent evaporation method was used to prepare microparticles, which was adapted from the process described by Beck et al. (1979); a solution of PLGA in dichloromethane (DCM) was emulsified with an aqueous solution containing an emulsion stabilizer using a Silverson homogeniser (Silverson Machines, Chesham, Bucks). The resulting oil-in-water emulsion was stirred for 12–18 h under ambient conditions to allow solvent to evaporate and the microparticles were then collected by centrifugation. The microparticles were cleaned and freeze dried and the final product was stored in a desiccator below 25°C. The prepared microparticles were sized by laser diffractometry using a Malvern Laser sizer 2600D and sizes were expressed as volume mean diameter (VMD).

The objectives of the current investigations were to optimise the particle preparation process to produce particles of approx. 1 μm in mean diameter. In these studies, the effects of the following four formulation variables on particle size were investigated:

(i) Nature and concentration of emulsion stabilizer;

(ii) Concentration of the polymer solution: This was studied either by variation in the weight of polymer dissolved in a fixed volume of DCM (100, 300 and 500 mg PLGA in 5 ml DCM), or by variation in the volume of DCM in which a fixed weight of polymer was dissolved (150 mg PLGA dissolved in 2.5, 5, 7.5 and 10 ml DCM);

(iii) Ratio of organic/aqueous phase volumes: This was studied in two ways, by variation in the volume of the organic phase emulsified with a constant volume of aqueous phase (2.5, 5, 7.5 and 10 ml organic phase with 10 ml aqueous phase) and by variation in the volume of aqueous phase emulsified with a constant volume of organic phase (2.5 ml organic phase with 10, 25, 50, 75 and 150 ml aqueous phase). The concentrations of polymer in the organic phase and PVA in the aqueous phase were maintained throughout the investigation at 6 and 10% w/v, respectively;

(iv) Rate and duration of agitation during emulsification (stirring rates of 6800, 8500, and > 10000 rpm for 5, 10, and 30 min).

Results and Discussion

Nature and concentration of emulsion stabilizer

It was not possible to produce microparticles in the absence of a stabiliser. Of the stabilisers studied, polyvinyl alcohol (PVA), sodium dodecyl sulphate (SDS), cetyltrimethyl ammonium bro-

TABLE 1

The effect of stabiliser type and concentration on particle size

Stabiliser	Concentration (% w/w)	Particle size (VMD ± SD) (μm)
Cetyltrimethyl ammonium bromide	1.0	0.5 ± 0.0
	5.0	0.5 ± 0.0
Gelatin	1.0	50.8 ± 2.6
Methylcellulose	0.05	52.4 ± 1.9
	0.5	28.9 ± 11.5
	1.0	16.2 ± 2.3
Polyvinyl alcohol	1.0	2.5 ± 0.1
	5.0	2.2 ± 0.1
	10.0	1.0 ± 0.0
Sodium dodecyl sulphate	1.0	4.1 ± 0.4
	5.0	2.4 ± 0.2

Microparticles were prepared as follows: 150 mg PLGA 50:50 (22 kDa) was dissolved in 2.5 ml DCM and emulsified with 10 ml of the stabiliser at 9800 rpm.

mide (CTAB), methylcellulose and gelatin all resulted in successful preparation of microparticles. However, microparticles were not prepared successfully when Tween 80 or poloxamine 908 were used as stabilisers.

An increase in surfactant concentration resulted in a decrease in particle size in all cases, except with CTAB. With CTAB, there was no obvious relationship between stabiliser concentration and particle size (Table 1). The choice of stabiliser significantly affected particle size and for a 1% w/v surfactant concentration, microparticles were produced in the following rank order of decreasing particle size; gelatin > methylcellulose > SDS > PVA > CTAB.

CTAB resulted in the formation of the smallest particles, but the sample produced was a thick unmanageable gel. Therefore, PVA was selected as the stabiliser of choice for further studies, since it resulted in the preparation of particles in the desired size range (approx. 1 μm), when used at a concentration of 10% w/v. PVA has also been used previously in microparticle preparation by a number of researchers (Beck et al., 1979; Benita et al., 1984).

Concentration of the polymer solution

Increasing the concentration (weight) of PLGA dissolved in a fixed volume of DCM resulted in an increase in particle size (Table 2). The higher concentration of polymer in the sample may have led to an increased frequency of collisions, resulting in fusion of semi-formed particles. This would produce an overall increase in the size of the microparticles. Increasing the concentration of dissolved polymer also increased the viscosity of the organic phase, which may have reduced the efficiency of stirring of the solutions.

Microparticles were prepared using several co-polymer ratios and polymer molecular weights. For all polymers studied, the relationship described above between concentration (weight) of polymer in the solvent and particle size showed

TABLE 2

The effect of polymer solution concentration on particle size by variation in the weight of polymer dissolved in a set volume of DCM

Weight of polymer (mg)	Concentration of polymer solution (% w/v)	Particle size (VMD) (μm) (polymer composition)			
		50:50 (9 kDa)	50:50 (22 kDa)	75:25 (18 kDa)	85:15 (53 kDa)
100	2	3.5	3.4	4.2	–
300	6	9.5	9.1	7.8	5.7
500	10	10.4	13.3	12.3	12.7
1000	20	–	–	–	26.1

Microparticles were prepared as follows: a given weight (in mg) of PLGA was dissolved in 5 ml DCM and emulsified with 50 ml of 1.0% w/v PVA at > 10000 rpm for 5 min. NB: The use of 50 ml of 1.0% w/v PVA solution resulted in an overall increase in particle size compared to results in Tables 1, 3 and 4.

TABLE 3

The effect of polymer solution concentration on particle size by variation of DCM volume

Volume of organic phase (ml)	Concentration of polymer solution (% w/v)	Particle size (VMD ± SD) (μm)
10	1.5	2.2 ± 0.0
7.5	2.0	2.2 ± 0.1
5.0	3.0	2.2 ± 0.2
2.5	6.0	3.2 ± 0.3

Particles were prepared as follows: 150 mg PLGA was dissolved in a given volume (in ml) of DCM and emulsified with 10 ml of 10% w/v PVA at > 10000 rpm for 5 min.

the same trend. However, there was no apparent relationship between particle size and the copolymer composition or molecular weight.

An alternative method of increasing polymer concentration in the organic phase was also investigated for its effect on particle size. Dissolving a fixed weight of polymer (150 mg) in decreasing volumes of DCM resulted in a slight reduction in particle size. This was attributed to the increased viscosity of the organic phase, which may have caused a reduction of the efficiency of disruption of the oil phase (Table 3).

It can be concluded from these results that the actual weight of polymer dissolved in the organic phase affects particle size to a greater extent than simple variation of the polymer concentration in this phase.

Ratio of organic / aqueous phase volumes

Increasing the volume of the organic phase, while maintaining a constant volume of the aqueous (continuous) phase, did not result in variations in particle size. However, a reduction in particle size was observed if the volume of the aqueous phase was reduced (Table 4). A reduction in the aqueous phase volume from 150 to 75 ml resulted in a 40% reduction in mean particle size (14.76 to 8.36 μm). For the aqueous phase volumes studied (against a fixed volume of organic phase), a linear relationship was observed between particle size and aqueous phase volume (Fig. 2).

TABLE 4

The effect of organic : aqueous phase ratio on particle size

Volume of organic phase (ml)	Volume of aqueous phase (ml)	Phase ratio (organic: aqueous)	Particle size (VMD ± SD) (μm)
2.5	10	1:4	2.6 ± 0.1
5.0	10	1:2	2.9 ± 0.2
7.5	10	3:4	2.7 ± 0.1
10	10	1:1	2.9 ± 0.2
2.5	25	1:10	3.6 ± 0.1
2.5	50	1:20	5.0 ± 0.2
2.5	75	1:30	8.4 ± 0.7
2.5	150	1:60	14.8 ± 0.2

Microparticles were prepared as follows: various volumes (in ml) of 6% w/v PLGA in DCM were emulsified with different volumes (in ml) of 10% w/v PVA solution at > 10000 rpm for 5 min.

Rate and duration of agitation during emulsification

During particle preparation a Silverson homogeniser was used to emulsify the disperse and continuous phases. An increase in the rate of agitation resulted in a reduction in particle size (Table 5). This trend was apparent for all stirring times investigated (5, 10 and 30 min). For a constant speed of 6800 rpm, increasing the stir-

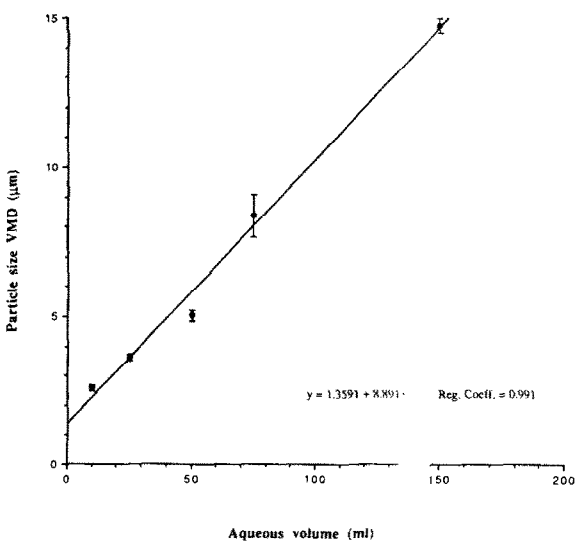

Fig. 2. Relationship between aqueous phase volume and particle size, VMD ± SD.

TABLE 5

The effect of stirring rate and duration on particle size

Rate (rpm)	Time (min)	Particle size (VMD ± SD) (μm)
6800	5	29.8 ± 0.9
6800	10	18.1 ± 1.5
6800	30	28.7 ± 0.3
8500	5	20.8 ± 1.3
8500	10	12.3 ± 1.4
8500	30	13.5 ± 0.1
> 10000	5	13.6 ± 0.5
> 10000	10	12.3 ± 0.2
> 10000	15	11.4 ± 0.0
> 10000	20	12.6 ± 0.1
> 10000	25	11.5 ± 1.2
> 10000	30	10.3 ± 1.8

Particles were prepared using the following method: 150 mg PLGA was dissolved in 2.5 ml DCM and emulsified with 25 ml of 1.0% w/v PVA. NB: The use of 50 ml of 1.0% w/v PVA solution resulted in an overall increase in particle size compared to results in Tables 1, 3 and 4.

ring time from 5 to 10 min resulted in a 40% reduction in particle size. However, increasing the stirring time to 30 min resulted in an increase in particle size, which may be attributed to particle aggregation. At 8500 rpm a 40% size reduction was also observed for samples stirred for 10 min rather than 5 min. However, stirring for 30 min did not result in a further reduction in particle size.

At a stirring speed greater than 10000 rpm, a series of samples were emulsified for 5, 10, 15, 20, 25 and 30 min. As stirring time increased up to 15 min a progressive, but small (15%) reduction in particle size was observed. Stirring for more than 15 min did not result in a further reduction in particle size.

These results indicated that stirring rate had a significant effect on the size of the microparticles, with higher rates of agitation resulting in the production of smaller particles. Nevertheless, at high speeds of stirring, the duration of agitation had no significant effect on particle size. Furthermore, at lower speeds (6800 and 8500 rpm) stirring for more than 10 min did not produce a reduction in particle size.

These investigations have provided an understanding of the effects of process parameters on particle size. Selection of the appropriate conditions has enabled the preparation of smooth, spherical PLGA microparticles 1–2 μm in mean size. There is potential for such formulations to be used in various aspects of oral and parenteral controlled release, following the entrapment of suitable active agents. In subsequent publications, the methods described in this paper for producing small microparticles will be adapted to allow entrapment of model peptides and proteins. These microparticles will be extensively characterised in vitro, before being used both orally and parenterally as drug and antigen delivery systems in small animal models.

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