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A novel preparation method for polymeric microparticles without the use of organic solvents

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Abstract

Microparticles (microcapsules and microspheres) of different chemical compositions (methacrylated dextran, methacrylated poly(ethyleneglycol) and gelatin) were prepared using a novel emulsion technique based on polymer–polymer immiscibility in aqueous solutions. The effect of polymer chemical composition and crosslink density on the properties of the microspheres was evaluated. Microparticles with a volume mean diameter ranging from 2.5 to 25 μm could be prepared in a reproducible way. Scanning electron microscopy revealed that non-porous and spherical particles were obtained for all preparations. Microcapsules with a single core were prepared using a double emulsion technology, based on the same principle of polymer–polymer immiscibility. These results demonstrate that this new emulsion technique without organic solvents can be used for the preparation of well-defined microparticles differing in size, morphology and chemical composition.

Keywords: Microparticle; Hydrogel; Dextran; Poly(ethylene glycol); Aqueous emulsion; Polymer–polymer immiscibility

1. Introduction

Polymeric microparticles are widely under investigation as pharmaceutical dosage forms for proteins and peptides. For the preparation of microspheres, many methods are based on emulsions formed by mixing an organic phase and an aqueous phase (Edman et al., 1980; O'Hagan et al., 1989; Kim et al., 1992; Lu et al., 1995). However, organic solvents are known to affect protein stability (Manning et al., 1989; Lu et al., 1995; Uversky et al., 1997).

In this paper, a novel, completely aqueous emulsion technique for the preparation of hydrogel microparticles based on polymer–polymer

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Table 1
The starting compositions of the water-in-water emulsions for the preparation of microspheres (rows A–H) and capsules (row I)

	Dispersed phase(s)	Continuous phase	Total Weight (g)
A	1% w/w Dex-MA DS 8	23% w/w PEG 10.000	5.5
B	1% w/w Dex-MA DS 8	29% w/w Pluronic F68	5.5
C	1.2% w/w PEG-MA_2	38% w/w Dextran T40	5.25
D	1.2% w/w PEG-MA_2	23% w/w Dextran T40 17% w/w $MgSO_4$	5.25
E	1.2% w/w PEG-MA_2	15% w/w Dextran T40 23% w/w $MgSO_4$	5.25
F	1.2% w/w PEG-MA_2	38% w/w $MgSO_4$	5.25
G	4% w/w Gelatin	12% w/w Poly(vinyl pyrrolidone) K90	2.5
H	4% w/w Gelatin	16% w/w Dextran T40	2.5
I	0.36% w/w PEG 10.000 (core phase) 0.75% w/w Dex-MA DS 8 (shell phase)	23% w/w PEG 4.000	5.0

Weight percentages are based on the composition of the total emulsion.

immiscibility is described. This aqueous polymer immiscibility occurs with many combinations of water-soluble polymers (e.g. combinations of dextran, poly(ethylene glycol) (PEG), poly(vinyl alcohol), poly(vinylpyrrolidone), gelatin, soluble starch or ficoll). The polymers stay in solution, but separate in two aqueous phases above a certain concentration (Albertsson, 1986). After emulsification, the polymer in the dispersed phase can be crosslinked to form a microparticle with a hydrogel character.

2. Materials and methods

Poly(ethylene glycol) with different molecular weights (4000–20000 g/mol) and potassium peroxodisulfate were obtained from Merck (Darmstadt, Germany). Dextran T40, poly(vinylpyrollidone) K90 and *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine were purchased from Fluka (Buchs, Switzerland). Gelatin A and anhydrous magnesium sulfate were from OPG (Utrecht, the Netherlands). Pluronic F68® was obtained from Serva (Heidelberg, Germany). Dimethacrylated PEG (PEG-MA_2; molecular weight 3400 g/mol) was from Shearwater Polymers (Enschede, the Netherlands). Methacrylated dextrans (Dex-MA) were synthesized and characterized according to Van Dijk-Wolthuis et al. (1995, 1997). Deoxygenated solutions were obtained by flushing with nitrogen for 10 min.

The general procedure for the preparation of dex-MA microspheres was as follows: deoxygenated aqueous solutions of dex-MA (0.28 g, 20% w/w) and PEG (5.2 g, 24% w/w; both phases in 0.22 M potassium chloride and 10 mM phosphate buffer pH 8.0) were vigorously mixed with a Vortex® for 1 min under an argon atmosphere. The resulting emulsion was allowed to stabilize for 10–20 min and subsequently, potassium peroxodisulfate (180 μl, 50 mg/ml) and *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine (100 μl, 20% v/v, pH neutralized with 4 M hydrochloric acid) were added. The mixture was incubated without stirring for 30 min at 37°C to polymerize the methacryloyl moieties in the dextran chains. The crosslinked dextran particles were purified by multiple washing and centrifugation steps. PEG microspheres were prepared by vigorously mixing of a deoxygenated aqueous solution of PEG-MA_2 (0.25 g, 25% w/w) and dextran or magnesium sulfate (40% w/w, 5 g in a scintillation vial of one solution or 2 g, 40% w/w dextran with 3 g, 40% w/w magnesium sulfate or 3 g, 40% w/w dextran with 2 g, 45% w/w magnesium sulfate) for 1 min with a Vortex® under an argon atmosphere. Crosslinking was done as described for dex-MA microspheres. Gelatin microspheres were obtained as follows: a gelatin solution (0.5 g, 20% w/w) was

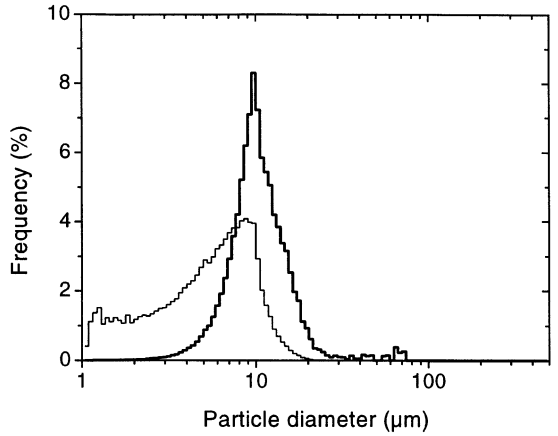


Fig. 1. Volume (bold) and number (thin) diameter distributions of dextran microspheres (dex-MA DS 8, PEG 10000 as continuous phase). Particle diameter distributions were obtained with a laser-light blocking technique (Accusizer™, model 770, Particle Sizing Systems, Santa Barbara, CA, and C770 software version 2.54).

added to a poly(vinylpyrollidone) or dextran solution (2 g, 15 or 20% w/w respectively) at 60°C in a glass-tube (10 ml). The solutions were emulsified

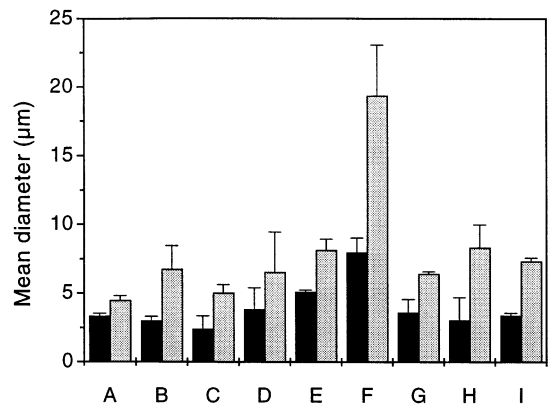


Fig. 2. Mean particle diameters of several preparations. Black and gray bars represent number (D[1,0] (Edmundson, 1967)) and volume mean (D[3,0] (Edmundson, 1967)) particle diameters, respectively (error bars are standard deviations, $n = 2–3$). A, Dex-MA DS 8 in PEG 20000. B, Dex-MA DS 8 in Pluronic®. C, PEG-MA_2 in 38% w/w dextran. D, PEG-MA_2 in 23% w/w dextran/17% w/w $MgSO_4$. E, PEG-MA_2 in 15% w/w dextran/23% w/w $MgSO_4$. F, PEG-MA_2 in 38% w/w $MgSO_4$. G, Gelatin in poly(vinylpyrrolidone). H, Gelatin in dextran. I, PEG 10000/Dex-MA DS 15 microcapsules. For the starting compositions of the emulsions, see Table 1.

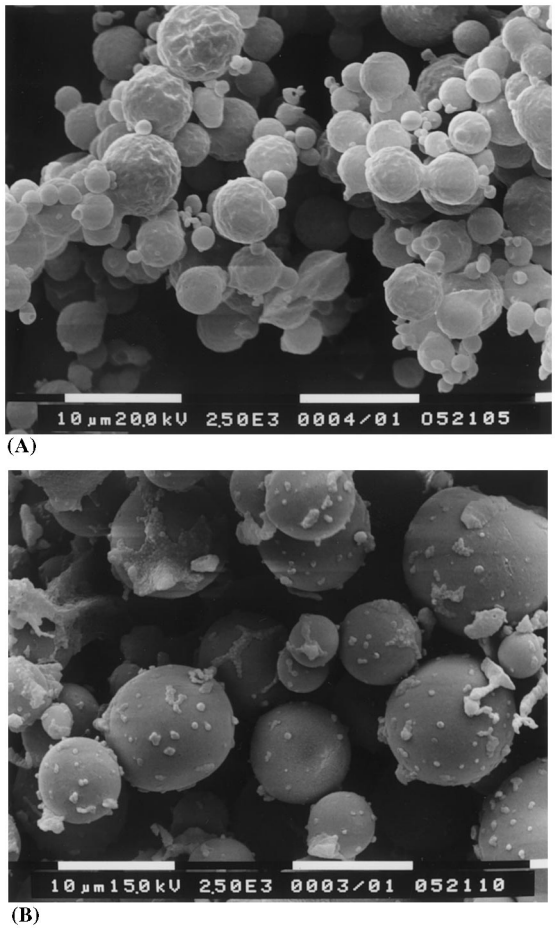
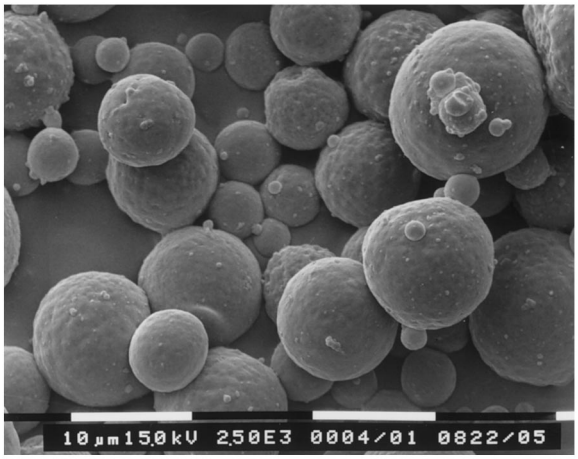


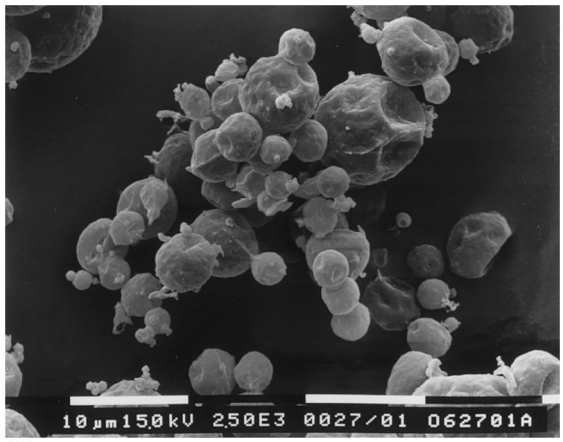
Fig. 3. SEM pictures of several dex-MA microspheres. A, Dex-MA DS 8 in PEG 10000. B, Dex-MA DS 15 in PEG 10000. The microparticles were washed five times with water, freeze-dried, spread over a carbon sticker and sputtered with gold. Pictures were taken with a Philips SEM 525 M scanning electron microscope (Eindhoven, The Netherlands).

with a Vortex® for 15 s and subsequently allowed to stand for 15 min at 60°C.

Microcapsules were prepared by this method as well: deoxygenated aqueous solutions of dex-MA (0.25 g, 20% w/w) and PEG (0.1 g, PEG 10000, 24% w/w) were vigorously mixed for 2 min with a Vortex® under an argon atmosphere. Immediately after vortexing, part of this primary emulsion (PEG in dex-MA, 0.25 g) was added to a PEG solution (M_w 4000, 4.75 g, 24% w/w). The mixture was gently homogenized yielding a double emul-



(A)



(B)

Fig. 4. SEM pictures of other microspheres. A, PEG-MA_2 in 15% w/w dextran/23% w/w $MgSO_4$. B, Gelatin in poly(vinylpyrrolidone).

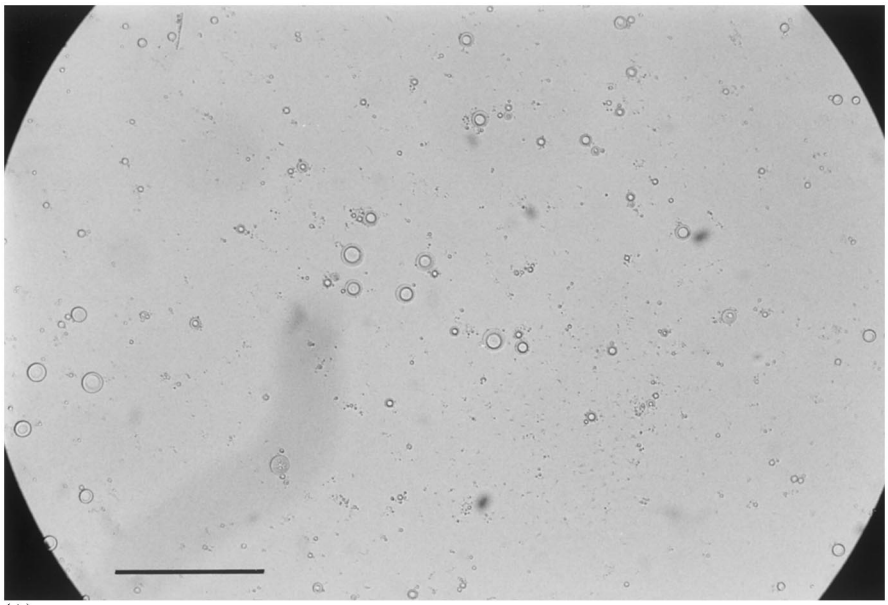
sion consisting of PEG/dex-MA/PEG. Next, the dex-MA was polymerized as described for the preparation of dex-MA microspheres. The starting compositions of all emulsions are listed in Table 1.

3. Results and discussion

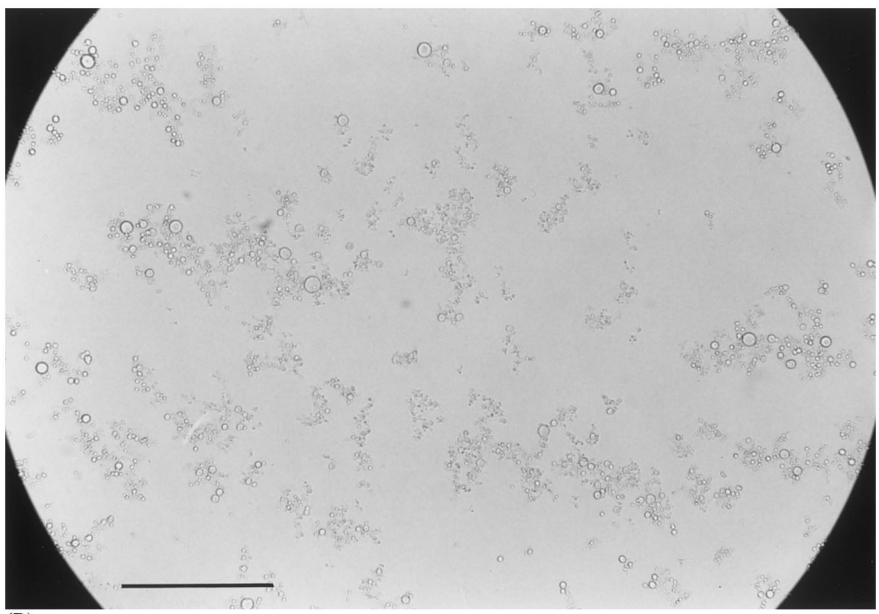
When aqueous solutions of PEG and dextran are mixed, a two-phase system can be obtained, depending on the molecular weight and the concentration of both polymers. After mixing, a water-in-water emulsion is obtained. Despite the high polymer concentrations used in some cases (up to 38% w/w), the viscosity of both dextran and PEG solutions remains below 250 mPa s. Therefore, the solutions are easy to handle, and relatively stable emulsions are readily formed. Microparticles can be prepared by crosslinking the polymer in the dispersed phase. This crosslinking can be established by radical polymerization of methacrylated dextran (dex-MA) emulsified in an aqueous PEG solution. When the polymerization was initiated during vortexing of the emulsion, irregularly shaped, aggregated particles were formed. Therefore, polymerization of the methacryloyl moieties was started after stabilizing the system for 10–20 min. The crosslinked dextran particles can be easily collected and purified by multiple washing and centrifugation steps. The preparations as described were all on a small scale (5 g), but the method was tested successfully on a 50 g scale as well. Fig. 1 gives a representative example of the volume and number diameter distribution of dex-MA microspheres prepared with PEG as continuous phase.

In Fig. 2, the number and volume mean diameters of several microparticles are shown. Microparticles with a narrow distribution (ratio between the volume and number mean diameter) are particularly found for dex-MA microspheres (varying from 1.2 to 1.8). Mean volume diameters can be tailored from 2.5 to 25 μm volume mean diameter by changing the shear forces, the molecular weights of each polymer, the DS (degree of substitution; the amount of methacrylates per 100 glucopyranose residues) of the dex-MA and volume ratio of both phases (Stenekes et al., 1998).

Fig. 2 also demonstrates that this emulsion technique for the preparation of microparticles can be used for several other polymer combinations. First, PEG can be easily substituted by Pluronic F68® as continuous phase, although a slightly higher Pluronic concentration had to be used in order to obtain a good phase separation (30% instead of 24% w/w). The mean diameters found for this system are higher as compared to



(A)

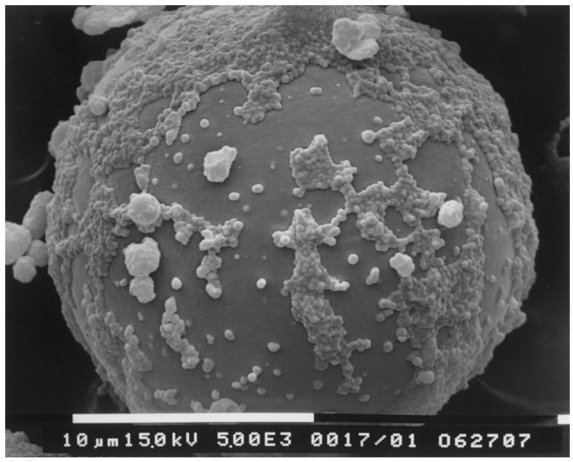


(B)

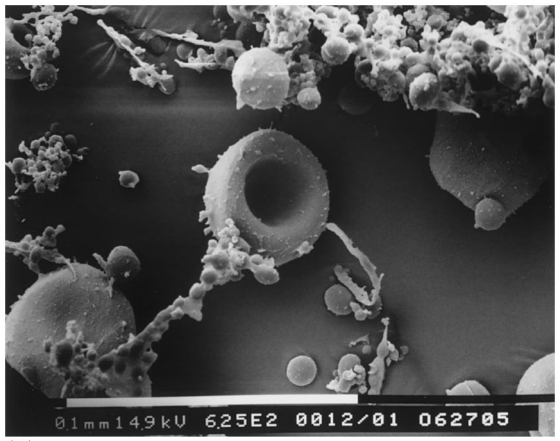
Fig. 5. Microscopy pictures of dex-MA microcapsules (PEG 10000/dex-MA DS 15/PEG 4000). A, Optical microscopy picture of microcapsules. B, Optical microscopy picture of similarly prepared microspheres (dex-MA DS 15 in PEG 4000). The bars represent 0.3 mm. C, A SEM picture of the surface of a microcapsule. D, A SEM picture of a broken microcapsule (continued on next page).

particles prepared using PEG 20000 as continuous phase (columns A and B).

Second, PEG microspheres can be formed by emulsification and polymerization of PEG-MA_2 in an aqueous dextran solution (column C). However, irregularly shaped particles with a relatively large size distribution were obtained. Probably, this can be ascribed to the PEG-MA_2 dissolved in



(C)



(D)

Fig. 5. (*Continued*)

the continuous dextran phase. The solubility of PEG-MA_2 in the dextran phase can be reduced by the addition of magnesium sulfate (Albertsson, 1986). This induced phase separation resulted in spherical particles with a narrow size distribution (columns D and E). The size of the PEG microspheres increases with a decreasing concentration of dextran, most likely due to the lower viscosity of the continuous phase (columns C–F). In principle, it is also possible to prepare PEG-MA_2 microspheres with only a sulfate salt in the continuous phase. However, rather large, aggregated particles are formed (column F). This can be ascribed to the low viscosity of the continuous phase. Third, small microspheres of gelatin can be obtained by emulsification of a gelatin solution in an aqueous solution of poly(vinylpyrrolidone) or dextran (columns G and H).

Interestingly, also microcapsules can be prepared using this emulsion technique (column I). For that purpose, a PEG/dex-MA/PEG double emulsion was created. After polymerization of the dex-MA, microcapsules were formed. The size distributions of these microcapsules were comparable to similar preparations without a core phase.

Fig. 3 shows scanning electron microscopy (SEM) pictures of dex-MA microspheres. A typical example of dextran microspheres with a low crosslink density (derived from dex-MA DS 8) made in a PEG 10000 solution as continuous phase is given in Fig. 3A. More or less spherical, non-porous microparticles are formed. Fig. 3B shows that microspheres with a higher crosslink density (derived from dex-MA DS 15) are more spherical and smoother, although small particles are found on their surface. Fig. 4A and B demonstrate that also in case of other polymers, like PEG-MA_2 (Fig. 4A) and gelatin (Fig. 4B), the particles remain non-porous. The gelatin particles are relatively irregularly shaped, whereas the PEG-MA_2 microparticles are smooth and spherical.

The morphology of microcapsules is presented in Fig. 5. Optical microscopy clearly reveals the presence of cores in the microcapsules (Fig. 5A), which are absent in similar microsphere preparations (Fig. 5B). Most of the microcapsules are mononuclear, having a uniform shell. SEM demonstrates that the surface of microcapsules is non-porous as well (Fig. 5C). The SEM picture of Fig. 5D shows the core of a microcapsule. This capsule might have been broken during the work-up procedure. Alternatively, the dex-MA shell phase polymerized during recombination of the PEG 10.000 core phase with the continuous PEG 4.000 phase.

This paper demonstrates that spherical, non-porous polymeric microparticles can be prepared using the principle of polymer–polymer immisci-

bility in aqueous emulsions. No organic solvents are used in this technology, which is very attractive for the preparation of protein-loaded microparticles.

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