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Short communication

The use of aqueous PEG/dextran phase separation for the preparation of dextran microspheres

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Abstract

A novel procedure to prepare dextran microspheres, without the use of organic solvents was developed. The method is based on phase separation which occurs in aqueous solutions of PEG and methacrylated dextran (dexMA). After stirring this two phase system a water-in-water emulsion is formed. When dexMA forms the discontinuous phase, dextran microspheres can be obtained by polymerization of the methacryloyl groups attached to dextran. The aim of this study was to gain insight into the formulation parameters that affect the particle characteristics. Therefore, it was necessary to establish dexMA/PEG/water phase diagrams. Lower polymer molecular weights and higher degrees of MA substitution resulted in less pronounced phase separation (binodal shifts to higher concentrations). The volume weight mean microsphere diameter varied between 2.5 and 20 μm, depending on the viscosities of both phases and the PEG/dexMA volume ratio. A more viscous continuous phase and/or a less viscous discontinuous phase resulted in smaller microspheres. Furthermore, the particle size increased with decreasing PEG/dexMA volume ratios. The particle characteristics, like cross-link density, initial water content and size can be tailored by adjusting the formulation parameters.

Keywords: Dextran microspheres; Organic solvent-free; Methacrylated dextran

Dextran hydrogels are interesting systems for the controlled release of proteins (Hennink et al., 1997; Van Dijk-Wolthuis et al., 1997a). For therapeutic applications of these protein-loaded gels,

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injectable dosage forms are preferred. An attractive method to prepare polymeric microspheres in an all-aqueous system, avoiding the use of organic solvents, is based on the phenomenon that in aqueous two-polymer systems phase separation can occur. This method is described in two recent publications of our group (Franssen and Hennink, 1998; Stenekes et al., 1998). This paper gives a summary of the main results obtained so far.

In a ternary system consisting of two water-soluble polymers (e.g. dextran and PEG) and water, liquid–liquid phase separation can occur (Fig. 1). From a thermodynamic point of view, phase separation in these systems occurs when the change in Gibbs free energy (ΔG_{mix}) is positive:

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix} > 0$$

where ΔH_{mix} is the enthalpy of mixing, T is the absolute temperature and ΔS_{mix} is the entropy of mixing. When the gain in entropy of mixing is not large enough to compensate for the repulsive dextran–PEG interaction enthalpy, mixing of the two polymers is thermodynamically not favorable and phase separation occurs.

The phase diagrams for PEG with methacrylated dextran (dexMA, prepared as described by

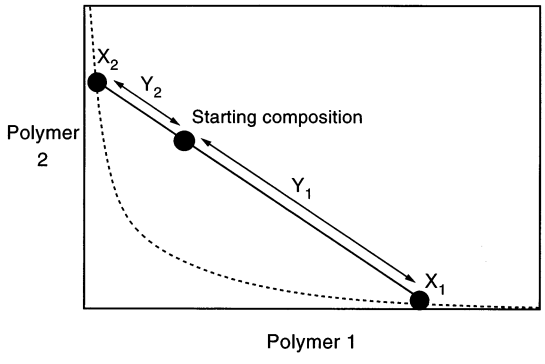

Fig. 1. Phase diagram of a water/PEG/dextran ternary system. When the starting-composition is below the binodal (--- -), a one phase system is present, whereas above the binodal, two coexisting phases are formed: one enriched in polymer 1 (composition x_1) and the other enriched in polymer 2 (composition x_2). x_1 and x_2 are connected via a tie-line (—). All systems prepared using starting-compositions on the same tie-line separate into phases of constant composition. For a given starting- composition, the volume ratio of the coexisting phases x_1/x_2 equals y_2/y_1.

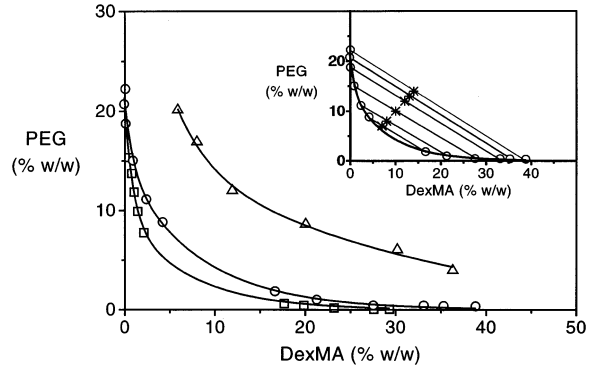

Fig. 2. Phase diagrams for PEG 10.000 and methacrylated dextran (dexMA) 40.000; DS 0 (□), 10 (○), 30 (△). The insert gives the phase diagram for PEG 10.000 and dexMA 40.000 (DS 10) including tie-lines and starting composition (*).

Van Dijk-Wolthuis et al., 1995, 1997b) were determined experimentally by choosing a number of starting compositions, for which phase separation was expected. These systems were vigorously shaken for 1 min and subsequently centrifuged. From the two phases a sample of about 1 ml was taken and analyzed for the PEG and dextran concentration using GPC analysis (Stenekes et al., 1998).

The microspheres were prepared as follows: aqueous solutions of PEG and dexMA in 0.22 M KCl were flushed for 10 min with nitrogen and subsequently transferred into a scintillation vial (total weight 5 g). The two phase system was vigorously mixed for 60 s to create a water-in-water emulsion. Next, the emulsion was allowed to stabilize for 15 min (ambient conditions), followed by the addition of TEMED (100 μl, 20% v/v, adjusted to pH 7 with 4 M HCl) and KPS (180 μl, 50 mg/ml). This system was incubated for 30 min at 37°C to polymerize the methacryloyl groups coupled to the dextran chains. The particle size and size distribution was measured with a laser light blocking technique (Accusizer™).

In Fig. 2, the phase diagrams for the ternary systems of water, PEG 10.000 and dex(MA) 40.000 with different degrees of substitution (DS = 0, 10, 30) are presented. Interestingly, the binodal shifts to higher PEG/dex concentrations with an increasing degree of MA substitution. Apparently, the PEG/dexMA interaction parame-

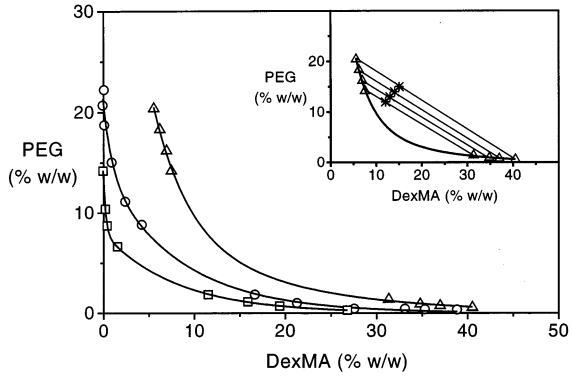

Fig. 3. Phase diagrams for PEG 10.000 with methacrylated dextran (dexMA) 6.000 (△), dexMA 40.000 (○) and dexMA 220.000 (□) (all DS 10). The insert gives the phase diagram for PEG 10.000 and dexMA 6.000 (DS 10) including tie-lines and starting-compositions (*).

ter is decreasing with an increasing degree of MA substitution (smaller ΔH_{mix}) indicating a favorable interaction between the methacryloyl groups and PEG. In Fig. 3, the phase diagrams for PEG 10.000 and dexMA (DS 10) with varying molecular weights are presented. As expected, the binodal shifts to higher concentration with decreasing molecular weight of dexMA, because at a fixed concentration, a lower molecular weight corresponds with more molecules per volume and thus a larger ΔS_{mix}. For phase diagrams of PEG with varying molecular weight and dexMA 40.000 (DS 10) the same trend was observed (results not shown).

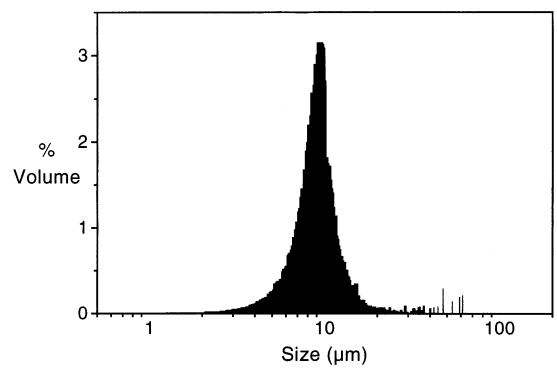

Fig. 4. Volume weight diameter distribution of dextran microspheres (starting composition: 1% w/w methacrylated dextran (dexMA) 220.000 (DS 10) and 28.5% w/w PEG 10.000) prepared using the water-in-water emulsion technique.

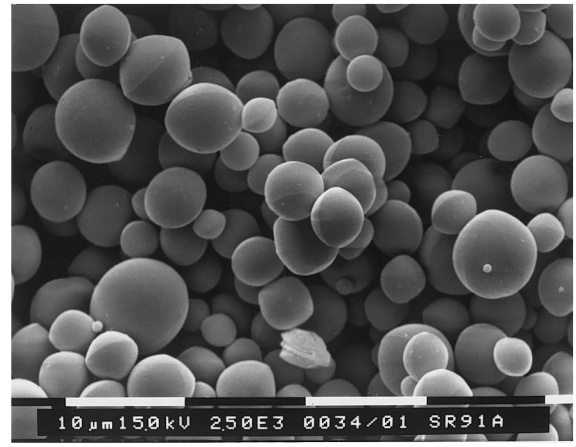

Fig. 5. Scanning electron microscope (SEM) picture of dextran microspheres (dexMA 220.000, DS 10, 63% water).

The microspheres prepared typically have a particle size distribution as shown in Fig. 4. In Fig. 5, a scanning electron microscope (SEM) picture of a representative microsphere batch is presented (dexMA 220.000, DS 10, 63% water).

To study the effect of the formulation parameters on the particle characteristics, microspheres were prepared at a fixed dexMA concentration of the discontinuous phase (36% w/w dexMA) and a fixed PEG concentration of the continuous phase

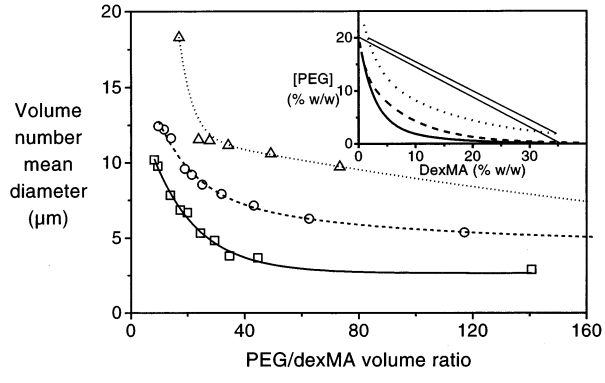

Fig. 6. Microsphere volume weight mean diameter vs PEG/methacrylated dextran (dexMA) volume ratio for dexMA 40.000 (DS 10) and PEG 4.000 (· · ·Δ· · ·), PEG 10.000 (--○- -) and PEG 20.000 (–□–). The insert gives the corresponding phase diagrams and tie-lines.

(21% w/w PEG), but with varying volume ratios of the two phases and varying PEG molecular weights. This was established by selecting starting compositions on tie-lines of the different phase diagrams (Fig. 6, insert). Fig. 6 shows for three PEG molecular weights the effect of the PEG/dexMA volume ratio on the particle size of the formed dextran microspheres. At PEG/dexMA volume ratios >40, the average microsphere diameter was not dependent on the volume ratio. However, at volume ratios <40, the particle size increased with decreasing volume ratio. Fig. 4 also shows that (at fixed concentrations in the coexisting phases), larger dextran microspheres were formed when lower PEG molecular weights were used. When the dextran molecular weight was varied, the opposite trend was observed: larger microspheres were obtained with increased dextran molecular weight (results not shown). The water content of the microspheres can be tailored by selecting an appropriate tie-line length (longer tie-line results in higher % w/w dex in the dextran phase, so a lower initial water content in the dextran microspheres).

So, dextran microspheres with tailored characteristics (size, equilibrium water content) can be prepared using a novel process based on polymer–polymer immiscibility in an aqueous system. Presently, the release of model-proteins from degrading and non-degrading microspheres is being studied.

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