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Equilibrium water content of microspheres based on cross-linked dextran

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Abstract

In this paper a method is presented to determine the equilibrium water content of microspheres with a hydrogel character based on cross-linked dextran. The water content was established by determination of the increase in blue dextran concentration after incubation of this solution with dried microspheres. An excellent correlation between the actual and predicted water contents was observed for microspheres with a moderate to high cross-link density. On the other hand, for particles with low cross-link density, the equilibrium water content was higher than predicted. This could be fully ascribed to swelling of the microspheres. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Aqueous two phase system; Dextran; Microspheres; Water content

1. Introduction

Biodegradable hydrogels based on cross-linked dextrans have been shown to be attractive systems for the controlled release of proteins (Hennink et al., 1996, 1997; Franssen et al., 1997; Van Dijk-Wolthuis et al., 1997). It has been demonstrated that in gels with large mesh sizes, the release kinetics of an encapsulated protein was dependent on the water content of the hydrogel. On the other hand, in gels where the average mesh size was smaller than the protein diameter, screening occurred. Macroscopic gels with a degree of substitution (DS, the number of methacryloyl groups per 100 glucopyranose residues) larger than ten were dimensionally stable and did not swell, whereas gels with a DS smaller than ten showed water uptake once exposed to aqueous buffer solutions (Hennink et al., 1996).

Besides macroscopic hydrogels, we also demonstrated that dextran microspheres can be prepared, using a novel, all-aqueous procedure based on the phenomenon of phase-separation in aqueous systems containing two polymers (Franssen and Hennink, 1998; Stenekes et al., 1998). In a previous study, we investigated the effect of the formulation parameters on the parti-

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cle characteristics (Stenekes et al., 1998). The size of the dextran microspheres was dependent on the viscosity of both phases and the volume ratio between the two phases. Tailoring of the initial water content, in theory, is possible by selecting appropriate preparation conditions, using the phase-diagram (Albertsson, 1986) of poly(ethylene glycol) (PEG) and methacrylated dextran (dexMA) (Stenekes et al., 1998).

The aim of the present study was to determine the actual equilibrium water contents of dextran microspheres and to compare these values with the predicted ones.

2. Materials and methods

PEG 10.000 (M_w 12 000 g/mol; M_n 8700 g/mol) and potassium peroxodisulfate (KPS) were obtained from Merck (Darmstadt, Germany). Dex 40.000 (M_w 38 800 g/mol; M_n 16 400 g/mol) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Fluka (Buchs, Switzerland). M_w and M_n refer to the weight and the number average molecular weight, respectively, and were determined by GPC. Blue dextran 2000 (apparent molecular weight $\approx 2\,000\,000$ g/mol) was obtained from Pharmacia Biotech AB (Uppsala, Sweden).

DexMA was prepared and characterized essentially as described previously (Van Dijk-Wolthuis et al., 1995, 1997). Batches with degrees of substitution (DS: the number of MA groups per 100 dextran glucopyranosyl monomer units) of 5, 10 and 20 were used. Dextran microspheres were prepared using a water-in-water emulsion as described by Stenekes et al. (1998). The cross-linked dextran particles were collected and purified by multiple centrifugation and washing steps. Next, the microspheres were frozen, while rotating, in liquid nitrogen and subsequently freeze dried (type Christ, Alpha 1-2).

Macroscopic hydrogels (diameter ~ 0.9 cm, height ~ 2 cm) were prepared by adding 75 μl TEMED (20% v/v, adjusted to pH 7 with 4 M HCl) and 135 μl KPS (50 mg/ml) to 1.29 g of a dexMA solution of desired concentration in an Eppendorf vial. Polymerization was performed at 37°C for 30 min. Thereafter, the gels were placed in vials containing 15 ml reversed-osmosis water at room temperature. At regular time intervals, the weight of the gels was determined and used to calculate the swelling ratio (defined as W_t/W_0, in which W_t is the weight of the gel at time t and W_0 is the initial weight of the gel).

3. Results

Dextran microspheres with different water contents and degrees of methacrylate substitution were prepared. Several attempts to establish the water content of microspheres are described in the literature, e.g. by determination of the difference in total volume of wet and dry microspheres (Kesenci and Piskin, 1998), or the difference in total weight either with (Shukla et al., 1991; Murat Elçin, 1995) or without blotting (Atkins et al., 1993). In another study, the wet microspheres are filtered and the change in filter mass was determined (Wang and Wu, 1998). However, in our hands these methods gave irreproducible results, because it is difficult to discriminate between water that is present in the microspheres and water present between the microspheres.

Another approach for the determination of the solvent content of microspheres is based on their change in size upon swelling. The increase in size can be determined using static and dynamic light scattering techniques (Rodriguez et al., 1994) as well as with microscopic methods (Fukuda et al., 1991; Kim and Lee, 1992). The equilibrium water content of the microparticles can be derived from the difference between the average diameter in the dry and swollen state. A prerequisite for using this approach is that the dry dextran microspheres are non-porous. It was shown using mercury intrusion porosimetry (Carli and Motta, 1984; results not shown), B.E.T. measurements (Lowell and Shields, 1987; results not shown) and scanning electron microscopy (Fig. 1) that the particles were essentially non-porous. However, evaluation of the average diameter of the dry and swollen particles using laser light blocking and laser diffraction techniques resulted in large experimental errors in the water contents of the microspheres.

We therefore developed a simple and elegant method to determine the water content of dextran microspheres using an aqueous solution of blue dextran (initial concentration 6 mg/ml). This compound is too large to penetrate into the microspheres. Therefore, its concentration will increase due to absorption of water by the dry microspheres. The hydrated microspheres were separated from the solution by centrifugation (3 min, Eppendorf centrifuge) and the increase in dextran blue concentration in the supernatant can easily be quantified spectrophotometrically ($\lambda = 610$ nm; linear range: 0–1000 μg/ml, $R^2 = 0.9999$). This method was validated by using different amounts of microspheres and by varying the swelling time (Tables 1 and 2, respectively).

From Table 1 it appears that the experimentally determined water content was independent of the amount of microspheres added, when 25 mg or more was used. Table 2 shows that the dry microspheres were rapidly hydrated: no significant differences in water content were observed when the incubation time was varied from 5 to 4000 min. Throughout the remaining experiments, 40 mg microspheres were used and these were incubated for 1 h to rule out effects of the amount of microspheres and the incubation time on the resulting equilibrium water contents.

Furthermore, it was demonstrated that no aspecific interactions between blue dextran and the microspheres occurred (e.g. adsorption and/or absorption was marginal), since after one washing and centrifugation step, the microsphere pellet was colorless, indicating that no blue dextran had penetrated into the microspheres.

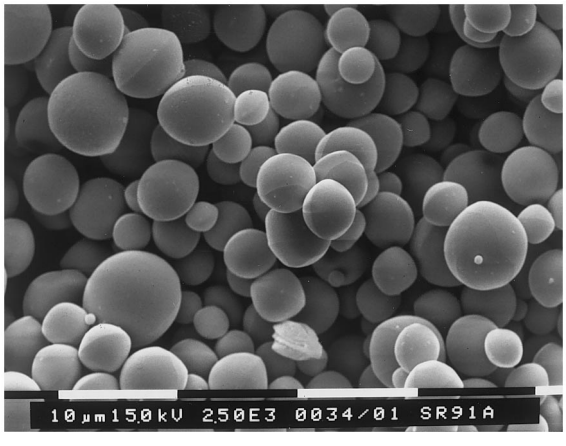

Fig. 1. Representative SEM picture of dextran microspheres.

Table 1
Water content of dextran microspheres (DS 5) as a function of the amount of microspheres added to the aqueous blue dextran solution (400 μl, 6 mg/ml)[a]

Predicted % H_2O (w/w)	Microspheres (mg)	% H_2O (experimental)
70	10.0	79.2
70	29.2	76.2
70	52.1	76.1
70	55.5	76.0
70	76.6	75.4
50	9.4	65.5
50	26.4	68.0
50	48.8	67.6
50	47.9	66.7
50	79.2	67.2
30	11.1	67.1
30	25.2	63.2
30	46.8	62.7
30	67.2	61.3
30	87.9	62.4

[a] Incubation time 1 h.

Table 3 shows the water contents of different batches of dextran microspheres. It is obvious that the water contents predicted by the phase-diagram corresponded very well with the experimentally determined values for microspheres with DS 10 or 20 and water contents of 70 and 50%. On the other hand, for expected water contents of 30%, equilibrium values of $\sim 50\%$ were obtained.

To investigate this discrepancy, we experimentally determined the tie-lines of the phase-diagrams at lower water contents, using the GPC

Table 2
Water content as function of swelling time (40 mg microspheres; DS 5; predicted water content 50%).

Time (min)	% H_2O (experimental)
5	67.5
15	67.3
60	66.6
150	66.0
4000	68.3

Table 3
Water contents (predicted and experimentally determined) of different batches of dextran microspheres[a]

DS	Predicted % H_2O (w/w)	% $H_2O \pm$ S.D. (experimental)
5	70	75.7 ± 0.1
5	50	67.3 ± 1.0
5	30	62.6 ± 0.2
10	70	65.9 ± 1.2
10	50	52.9 ± 0.1
10	30	54.8 ± 0.6
20	70	67.2 ± 1.4
20	50	51.9 ± 0.3
20	30	52.5 ± 0.8

[a] Incubation of 1 h, 40 mg microspheres.

method described earlier (Stenekes et al., 1998). As demonstrated in Fig. 2, the STL (the slope of the tie-line) becomes steeper with increasing concentrations of both PEG and dextran. This results in higher water contents (lower dextran concentrations in the dextran phase) than expected and can very well explain the discrepancy between found and predicted water contents (Table 3).

Table 3 also demonstrates that the equilibrium water contents for microspheres with DS 5, give higher values than those predicted by the phase-diagram. A likely explanation is that gels with DS 5 are not dimensionally stable, as demonstrated before (Hennink et al., 1996). A macroscopic hydrogel with DS 5 and an initial water content of 70% was prepared and its swelling behavior was established. The equilibrium swelling ratio was 1.28, which corresponds with an equilibrium water content of 76%. Interestingly, for microspheres with the same composition, an equilibrium water content of 76% was also found (Table 3), which explains very well the difference between the actual and predicted water contents.

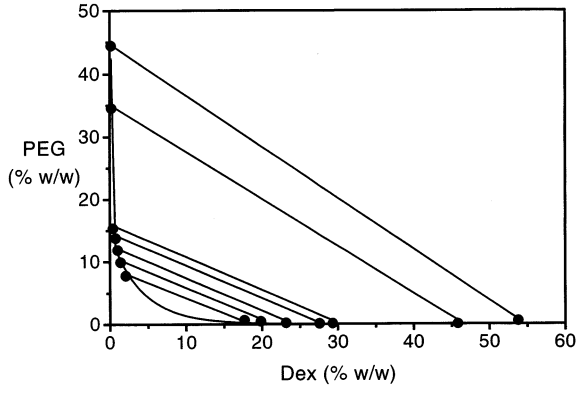

Fig. 2. Phase-diagram also including tie-lines at starting compositions far from the critical point.

4. Conclusions

In conclusion, this paper describes a very simple and elegant method to accurately determine the equilibrium water content of microspheres with a hydrogel character based on cross-linked dextrans. This method is based on the exclusion of a high molecular weight compound (blue dextran) from the microspheres and absorption of water by the dry microspheres. Its working range depends on the volume and concentration of dextran blue solution as well as the amount of microspheres used and the amount of water adsorbed by these microspheres: the water absorption should result in a significant increase in dextran blue concentration. It can be envisaged that this method is universally applicable for different types of microspheres with a hydrogel character.

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