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Degradable dextran microspheres for the controlled release of liposomes☆

R.J.H. Stenekes [a,b], A.E. Loebis [a], C.M. Fernandes [a], D.J.A. Crommelin [a,b], W.E. Hennink [a,*]

[a] *Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), University of Utrecht, PO Box 80082, 3508 TB Utrecht, The Netherlands*

[b] *OctoPlus B.V., PO Box 722, 2300 AS Leiden, The Netherlands*

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Abstract

A novel delivery concept based on the encapsulation of liposomes in biodegradable dextran microspheres was developed. The microspheres were prepared using a two-phase system, consisting of water/poly(ethylene glycol), and water/methacrylated dextran. Liposomes were encapsulated almost quantitatively and in their intact form, and were released with full preservation of their integrity. The effects of microsphere water content, degree of methacrylate substitution, and type of dextran derivative used on the release rate were investigated. The release of the liposomes from the dextran microspheres was fully controlled by the degradation rate of the spheres. This resulted, after a lag time, in a pulsed release of the liposomes from relatively rapidly degrading microspheres. On the other hand, slower degrading microspheres resulted in sustained release of liposomes over 100 days. The degradation rate of the dextran microspheres, in turn, depended on the water content, the degree of methacrylate substitution, and type of hydrolytically sensitive spacer present in the cross-links.

Keywords: Degradable; Dextran microspheres; Controlled release; Liposomes

Liposomes have been studied for many years as a carrier system for drugs (Storm and Crommelin, 1998). The bilayer characteristics and the physicochemical nature of the drug determine the release of the drug from the liposome. After subcutaneous or intramuscular injection, the duration of a liposome associated drug can be prolonged to a few weeks at most (Kadir et al., 1992).

Other carrier systems gaining increasing attention are polymeric hydrogels (Kamath and Park, 1993). The use of biocompatible (Cadée et al., 2000), biodegradable dextran-based hydrogels as a protein-releasing matrix (Hennink et al., 1996) allows well-controlled release of encapsulated drugs. A technique to prepare dextran microspheres in an aqueous two-phase system was de-

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* Corresponding author. Tel.: +31-30-2537306; fax: +31-30-2517839.

E-mail address: w.e.hennink@pharm.uu.nl (W.E. Hennink).

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veloped to facilitate parenteral administration (Stenekes et al., 1998). However, it can be foreseen that release of low molecular weight drugs from these microspheres is difficult to control. Since low molecular weight drugs can be well entrapped in liposomes, the aim of our work was to design an injectable dosage form that can release intact drug-carrying liposomes, both in a pulsed and in a sustained way over a period of months. The results of this work are described in a recent publication (Stenekes et al., 2000) and are summarized in this paper.

DPPC:DPPG:Chol (10:1:10) and EPC:EPG (9:1) liposomes were prepared by lipid film hydration and extruded to obtain the desired size. Microspheres were prepared as follows (Stenekes, et al., 1998). An aqueous phase separated system consisting of poly(ethylene glycol) (PEG) and methacrylated dextran (dexMA, dexHEMA or dex(Lactate)$_2$HEMA, prepared as described previously; van Dijk-Wolthuis et al., 1997b,c; Cadée et al., 1999) in 10 mM Hepes buffer was vigorously mixed to create a water-in-water emulsion. After 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 methacrylate groups coupled to the dextran chains. Liposomes were encapsulated in the microspheres by dispersing the liposomes in the dextran solution prior to addition to the PEG solution. The subsequent steps in the microsphere preparation procedure were unchanged. The fraction of liposomes in the microspheres was determined by measuring the phosphate concentration in the microsphere pellet using the method of Rouser et al. (1970). The 'standard formulation' refers to a water content (Stenekes and Hennink, 1999) of the dextran phase of 70% (w/w), a PEG/dextran volume ratio of 40, a degree of methacrylate substitution (DS) of 10 (dexMA, for the encapsulation efficiency studies) or a DS of 8 (dexHEMA, for the release studies), a polymerization temperature of 37°C, and DPPC:DPPG:Chol (10:1:10) liposomes with a size of 0.18 μm.

Liposomes were entrapped almost quantitatively in the microspheres ($94 \pm 6\%$, $n = 5$; standard formulation). Fig. 1 presents the phase transition of the bilayer of EPC:EPG liposomes before and after encapsulation in microspheres, as monitored by differential scanning calorimetry (DSC). Obviously, the transition temperature of the liposomes (-23.4°C) did not change significantly upon encapsulation in microspheres (-23.1°C), indicating that the liposomal bilayer was not disturbed during encapsulation.

Furthermore, confocal fluorescence microscopy demonstrated that the liposomes were homogeneously distributed over the microspheres and that a membrane intercalating probe (rhodamine–PE) was not leached from the liposomes (Fig. 2).

Fig. 3 shows a typical example of the release profile of liposomes from three independently prepared batches of biodegradable dextran microspheres (standard formulation). The release was derived from the amount of phosphate detected in the incubation buffer. During the first 17 days, about 10% of the liposomes were released, probably due to liposomes located close to the surface of the microspheres. The remaining liposomes are entrapped in the meshes of the hydrogel network. Due to hydrolysis of the carbonate esters in the cross-links of the dextran network, the mesh size will increase in time. Once the meshes in the hydrogel are larger than the size of the liposomes, they will diffuse out of the microspheres. These combined effects of initial entrapment of lipo-

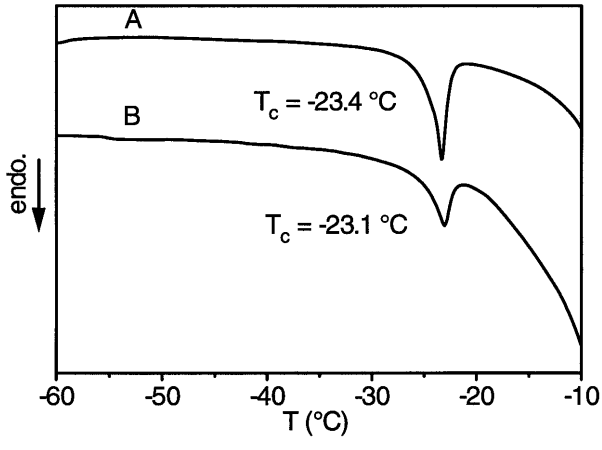

Fig. 1. DSC thermogram of EPC:EPG (9:1 molar ratio) liposomes (A) and EPC:EPG (9:1 molar ratio) liposomes entrapped in dexMA microspheres (B) (standard formulation).

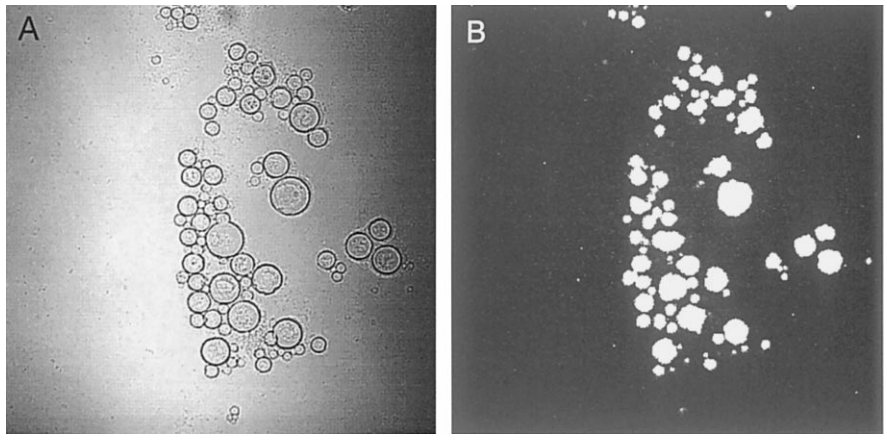

Fig. 2. Light microscope (A) and confocal laser-scanning microscope (B) picture of microspheres containing rhodamine–PE-labeled liposomes (same area). Formulation: DS 8, 50% water.

somes in the hydrogel network followed by a relatively rapid release upon gel degradation are most likely responsible for the observed pulsed release profile. Non-degradable dexMA microspheres showed a marginal fraction of liposomes released in the time frame studied (Fig. 3). In Fig. 4, the release of free and total calcein is presented for calcein containing liposomes in microspheres. It is obvious that hardly any free calcein was released for more than 18 days. However, after treatment of the samples with a liposome destabilizing agent (triton X-100), a pulsed release of calcein was observed. This shows that, after being released from the microspheres, calcein is still present in the aqueous core of the liposomes, where the fluorescence of this compound is quenched. This clearly demonstrates that the integrity of the liposomes is fully preserved after encapsulation and release. Moreover, DLS measurements showed that the size of the liposomes after release (0.20 and 0.10 μm with DP 0.14 and 0.19, respectively) is similar to the particle size before encapsulation in the microspheres (0.18 and 0.10 μm with DP 0.10 and 0.05, respectively).

Fig. 5 shows the release of liposomes from microspheres varying in DS, initial water content and type of hydrolyzable group in the cross-links. Obviously, the lag-time decreased with DS (13 and 18 days for DS 5 and 8, respectively), which can be ascribed to the decreasing degradation rate of dexHEMA gels when lowering the DS. For gels with a DS of 8, the initial water content had a large effect on the release of the entrapped liposomes. In microspheres with a high initial water content (70%), the liposomes were released in a pulsed manner after a delay time of 18 days. In contrast, particles with a lower initial water content (50%) showed a more sustained release pattern for the entrapped liposomes (up to 100 days) after a slightly longer delay time (20–23 days). Fig. 5 also shows that the release of liposomes from dex(Lactate)$_2$HEMA microspheres (DS = 4)

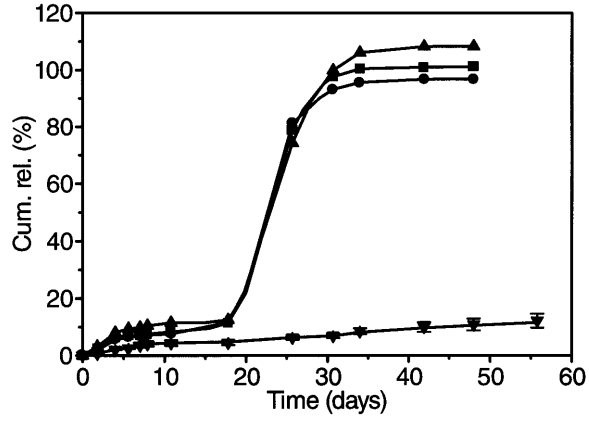

Fig. 3. Liposome release from three individually prepared biodegradable dexHEMA microsphere batches (●, ■ and ▲) and non-degradable dexMA microspheres (▼) (standard formulation) at pH 7.2.

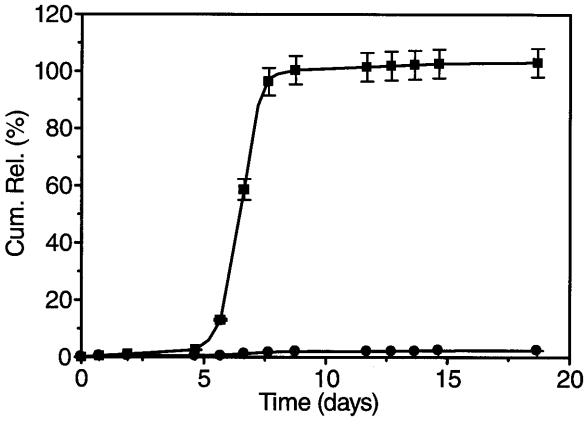

Fig. 4. The release of calcein at pH 8.0 before (●) and after (■) treatment of the samples with triton X-100 using the standard formulation and calcein containing liposomes (0.22 μm).

was much faster than from dexHEMA based microspheres with similar DS (6 and 18 days, respectively), which can be explained by the fact that the lactate ester present in dex(Lactate)$_2$HEMA is more susceptible to hydrolysis than the carbonate ester present in dexHEMA (van Dijk-Wolthuis et al., 1997a). In conclusion, we designed a novel drug delivery system based on the encapsulation of liposomes in biodegradable dextran microspheres, which released the liposomes intact and in a controlled way over extended periods of time.

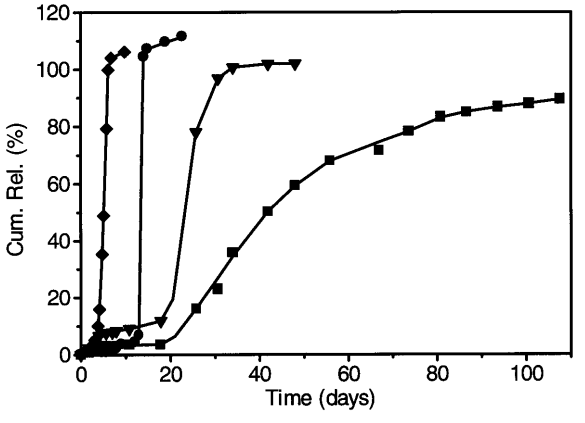

Fig. 5. The release of liposomes from dexHEMA microspheres at pH 7.2 using different formulations: (●) DS 5 and 70% water; (▼) DS 8 and 70% water; (■) DS 8 and 50% water, and the release of liposomes from dex(Lactate)$_2$HEMA microspheres; and (◆) DS 4 and 70% water.

Acknowledgements

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