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Enzymatically degradable nanoparticles of dextran esters as potential drug delivery systems

Kulthida Kaewprapan[a,b,c], Pranee Inprakhon[a], Emmanuelle Marie[c,1], Alain Durand[c,*]

[a] Department of Biotechnology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand
[b] Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok, Thailand
[c] Laboratoire de Chimie-Physique Macromoléculaire, UMR 7568 CNRS-Nancy University, ENSIC, BP 20451, 54001 Nancy Cedex, France

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ABSTRACT

Dextran decanoate esters with degrees of substitution (average molar ratio of ester functions to glucose repeat units) between 20 and 150% were used to form nanoparticles via nanoprecipitation technique. Particle size and colloidal stability of nanoparticles were found to depend on dextran concentration and degree of substitution. The colloidal stability of nanoparticle suspensions in sodium chloride solutions was improved by using a water-soluble dextran derivative as stabilizer. Enzymatic hydrolysis of ester bonds by porcine pancreatic lipase was demonstrated for highly modified dextran derivatives (up to DS = 150%). Complete degradation of low modified dextrans (DS up to 25%) by dextranase occurred within 7 days. Finally, encapsulation of lidocaine (as a model drug) into nanoparticles obtained with dextran esters (DS ranging between 21 and 150%) was investigated.

1. Introduction

Nanoparticles formulated from biopolymers have raised extensive interest for drug delivery applications over the past decades. Important parameters for effective nanoparticles for biomedical uses are biodegradability, biocompatibility, low cytotoxicity, high stability in different administration routes, high loading, specific site targeting and controlled drug release. A number of polymers have been reported to be efficient materials for nanoparticles preparation including synthetic polymers such as poly(lactic-co-glycolic acid), polylactide and polyalkylcyanoacrylates (Gomez-Gaete, Tsapis, Besnard, Bochot, & Fattal, 2007; Rouzes, Léonard, Durand, & Dellacherie, 2003; Wu, Dellacherie, Durand, & Marie, 2009), and natural polymers such as albumin, gelatin, chitosan and dextran (Lu, Tan, Hu, & Jiang, 2005; Qi, Xu, Jiang, Hu, & Zou, 2004; Tang, Dou, & Sun, 2006; Tseng et al., 2008). Amongst the different polysaccharides, dextran, a bacterial polysaccharide consisting essentially of α-1,6 linked D-glucopyranoside residues, is well known for its degradability by dextranase, biocompatibility and non-toxicity. The use of this biopolymer is widespread in medical and pharmaceutical fields. One proposed strategy was to covalently attach active molecules onto dextran macromolecules, which is the prodrug route (Dhaneshwar, Kandpal, Gairola, & Kadam, 2006; Heinze, Liebert, Heublein, & Hornig, 2006). Alternatively, the idea to attach hydrophobic groups along dextran chains in order to confer a capacity of self-organization at the nanometer scale for encapsulating hydrophobic active molecules has been also investigated. Nanoparticles were obtained from dextran modified with epoxides (Aumelas, Serrero, Durand, Dellacherie, & Léonard, 2007), cholic acid (Nichifor, Lopes, Carpov, & Melo, 1999) and fatty acid esters (Ge, Lu, Yang, & Liu, 2011; Hornig & Heinze, 2007) (Broaders, Grandhe, & Fréchet, 2011; Kaewprapan et al., 2011). Molecular parameters like average molar mass of dextran molecules, nature of grafted functional groups and degree of substitution (DS, average molar ratio of formed ester bonds to glucose repeat units in one macromolecule) were shown to influence nanoparticles diameter (Aumelas et al., 2007; Hornig & Heinze, 2007).

Apart from the need to develop suitable synthesis strategies for obtaining controlled colloidal carriers, other important aspects are the toxicity of all reactants/solvents used as well as the kinetics of degradation. In our previous work, we already reported the enzyme-catalyzed synthesis of dextran fatty esters (Kaewprapan, Tuchinda, Marie, Durand, & Inprakhon, 2007; Kaewprapan et al., 2011). Aliphatic hydrocarbon chains were grafted onto dextran backbone by lipase catalysis. Dextran esters with degrees of

* Corresponding author.
E-mail address: alain.durand@ensic.inpl-nancy.fr (A. Durand).
[1] Current address: ENS-Département Chimie, UMR 8640 CNRS-ENS-UPMC, 24 Rue Lhomond, 75005 Paris, France.

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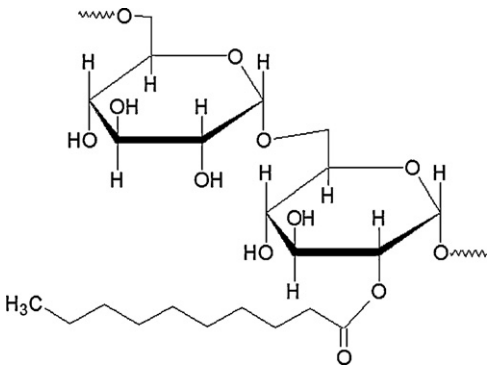

Fig. 1. Structure of dextran esters. Hydrocarbon tails are assumed to be randomly distributed within dextran chains.

substitution up to 150% were obtained. This was the first report of enzyme-catalyzed synthesis of dextran esters with long hydrocarbon chains and DS higher than 50%. Highly modified dextrans were soluble in THF or THF/water mixture, whereas low modified dextrans (DS < 15%) were soluble in water. Because of their solubility in water-miscible solvents, dextran esters with DS > 80% could be used to prepare nanoparticles by nanoprecipitation method and preliminary experiments demonstrated the feasibility of the process (Kaewprapan et al., 2011).

The aims of this study were to use a series of dextran esters obtained by enzyme-catalyzed transesterification with DS covering a wide range (between 20 and 150%) for preparing nanoparticles and to evaluate their suitability for drug delivery applications in terms of size, colloidal stability, encapsulation capacity and enzymatic degradation.

2. Experimental

2.1. Materials

Native dextran was obtained from Pharmacosmos (Holbaek, Denmark) (manufacturer data: M_n = 26,000, M_w = 40,000). Dextran decanoate was synthesized by enzyme-catalyzed reaction of vinyl decanoate with dextran T40 in dimethyl sulfoxide as described previously (Kaewprapan et al., 2011). Degree of substitution (DS) was defined as the average number of grafted decanoate groups (C10) per 100 units of glucose of dextran backbone. The chemical structure of dextran decanoate is shown in Fig. 1. Various degrees of substitution were obtained after progressive fractionation by ethyl acetate, methanol and water. Porcine pancreatic lipase type II, dextranase from *Penicillium* sp. were purchased from Sigma. Other products were analytical grade purchased from Sigma–Aldrich and used as received.

2.2. Methods

2.2.1. Preparation of nanoparticles

Modified dextrans were dissolved at various concentrations (5, 10 and 20 g/L) in 5 mL of either THF or THF/water mixture (90/10, v/v). The solution was added drop-wise into 10 mL of an aqueous solution with and without 1 g/L of low modified dextran under vigorous magnetic stirring. Nanoparticle suspensions were obtained after evaporation at 37 °C for 4 h.

2.2.2. Nanoparticles characterization

The intensity-average diameter (D_z) of nanoparticles was measured in 10^{-3} M NaCl at 25 °C by dynamic light scattering (DSL), using a Malvern High Performance Particle Sizer. Each diameter value was the average of three consecutive measurements. The standard deviation was ±5 nm. The "polydispersity index" (PDI) provided by HPPS was considered as a reasonable way to appreciate if the sample size distribution was roughly monomodal. PDI value estimates the quality of fitting the experimental DLS results using a monomodal particle size distribution. The higher the value, the worse the fit is. According to the furnisher and to our own experience, values lower than 0.3 correspond to particle size distributions that are reasonably monomodal while higher values correspond to very large distributions or even multimodal distributions. In any case, such samples cannot be considered as monodisperse (i.e. with a single size or almost). The majority of the samples considered in that work had PDI values lower than 0.3, which was considered as characteristic of monomodal samples.

2.2.3. Scanning electron microscopy was performed using a Hitachi S-2500 scanning electron microscope.

Stability of nanoparticle suspensions was investigated by measuring the dry extract of both precipitate and suspension after centrifugation at 1500 × *g* for 15 min at 25 °C. The stability of colloidal dispersions toward added electrolyte was assessed in NaCl by turbidimetry, as described by Long et al. (Long, Osmond, & Vincent, 1973). Experimentally, 100 μL of nanoparticles suspension were added to 3 mL of NaCl at various concentrations (10^{-3} to 4 M). The solution turbidity was measured in the range of 400–700 nm. The logarithmic plot of absorbance (A) versus wavelength (λ) gives the slope n indicating the size of particle. The critical NaCl flocculation concentration was determined at the n slope breaking as a function of NaCl concentration.

2.2.4. Degradation of nanoparticles by dextranase

About 10 mL of dextran solution (10 mg/mL) were incubated with dextranase (20 μg/mL, 41 U/mg) at 37 °C, pH 5.6. Samples were taken at 4, 24, 48 and 168 h of incubation time. Enzyme was then inactivated by heating at 95 °C for 10 min. The concentration of reducing oligosaccharides was determined as described by Franssen, Stenekes, and Hennink (1999). Typically, 2 mL of dextran solution were incubated with 3 mL of Sumner reagent for 15 min at 95 °C. After cooling, the absorbance was measured at 620 nm. Glucose solutions were used as references for calculating number of glucose unit in fragment.

2.2.5. Degradation of nanoparticles by porcine pancreatic lipase

Modified dextrans (10 mg/mL) were suspended in 20 mL of 20 mM phosphate buffer pH 7.7. The suspensions were incubated with 0.1 mg/mL pancreatic lipase from porcine (133 U/mg protein using olive oil as substrate) at 37 °C (Peng et al., 2010). One unit of lipase will hydrolyze 1.0 microequivalent of fatty acid from olive oil in 1 h at pH 7.7. The kinetic of fatty acid liberation was determined by titration with 0.05 M NaOH using Mettler Toledo DL50 titrator for 4 h (Kaewprapan et al., 2007). Hydrolysis extent was calculated based on the ratio of liberated fatty acid after hydrolysis to initial fatty acid in modified dextrans as shown in following equation.

$$\%\text{Hydrolysis} = \left(\frac{\text{liberated fatty acid}}{\text{initial fatty acid}}\right) \times 100$$

2.2.6. Encapsulation of lidocaine

Various amounts of lidocaine (0.5, 1.0, 2.5 and 5 g/L) were dissolved in the solution of modified dextran in THF (5 g/L). The solutions were added drop-wise into water with and without low modified dextran to form nanoparticles as mentioned previously. Amounts of encapsulated lidocaine were calculated from the lidocaine remaining in the supernatant after centrifugation of nanoparticles at 24,000 × *g* for 30 min, using Beckman Coulter (Optima L-100XP Ultracentrifuge) at 4 °C. The concentration of

Table 1
Solubility of modified dextrans in organic solvents (+ means fully soluble and – means partly insoluble, based on feed compositions corresponding to 1 g/L).

DS (%)	DMSO	Water	THF	THF/water (90/10, v/v)
15	+	+	–	–
25	+	–	–	+
80	+	–	+	–
150	+	–	+	–

lidocaine in supernatant was measured by spectrophotometer at 262 nm.

3. Results and discussion

3.1. Targeted structure and conditions for nanoparticles preparation

In the present work, nanoparticles were prepared from dextran decanoate esters with different degrees of substitution (Kaewprapan et al., 2011). These biopolymers were obtained by enzyme catalyzed transesterification reaction of vinyl decanoate on dextran. Dextran derivatives with DS ranging from 12 to 150% were recovered by fractionating crude reaction products using three solvents: ethyl acetate, methanol and water. These procedures are described in detail elsewhere (Kaewprapan et al., 2011, 2012).

First, the solubility in organic solvents of dextran with different degrees of substitution (DS) was studied (Table 1). Highly modified dextrans (DS > 80%) were soluble in tetrahydrofuran (THF). Modified dextrans with DS around 20–25% were soluble in a THF/water mixture (90/10, v/v). Modified dextrans with DS lower than 17% were water soluble. The solubility of highly modified dextrans in THF or THF/water mixture, water-miscible volatile solvents, offers the possibility of nanoparticle preparation by nanoprecipitation in water. This method has already been applied to prepare nanoparticles from modified dextrans or dextran graft copolymers in previous studies (Aumelas et al., 2007; Gavory et al., 2011; Ge et al., 2011; Hornig & Heinze, 2007; Ydens et al., 2005). As compared to other processes, nanoprecipitation has the advantage of being a simple, easily scalable and low energy procedure, avoiding the use of potentially degrading steps for active molecules.

Potentialities of dextran esters carrying long hydrocarbon chains and having degrees of substitution varying over a wide interval in the elaboration of drug delivery systems have never been reported. Thanks to enzyme-catalyzed modification of dextran, it was possible to design nanoparticles having an inner core with variable polarity according to the density of hydrocarbon tails grafting (DS). As for the surface of nanoparticles, it should be hydrophilic in order to provide a convenient colloidal stability to aqueous suspensions. Two possibilities were envisaged in that work. First, unmodified hydroxyl groups may from a hydrophilic layer at the surface of nanoparticles by self-organization of dextran ester macromolecules during nanoprecipitation. Obviously, to obtain an efficient hydrophilic barrier against particle aggregation, there should be enough non-reacted hydroxyl groups in macromolecules to generate a hydrophilic layer with a sufficient thickness. A second strategy was attempted, which was to prepare nanoparticles combining low and high DS dextran esters. The high DS polymer would form the inner core upon precipitation at the nanoscale while the low DS polymer would generate a hydrophilic corona by adsorption at the surface of self-generated nanoparticles.

In all reported experiments, high DS dextran esters (from 25 to 150%) were dissolved in organic phase (with concentrations ranging between 5 and 20 g/L) and low DS dextran ester (15%) if present, was dissolved in the aqueous phase (with a concentration equal to 1 g/L).

3.2. Effect of polymer concentration on average size and colloidal stability of nanoparticle suspensions

Three polymers with increasing DS were used to prepare nanoparticles. The conditions are gathered in Table 2. With all polymers, dextran nanoparticles in the range of 86–256 nm were obtained without the presence of any additional stabilizer in the aqueous phase. The smallest sizes were obtained when the polymer concentration in the organic phase was 5 g/L. Particle size increased with polymer concentration in organic solvent whatever the polymer used.

The colloidal stability of the suspensions was evaluated by considering the dry weight of remaining nanoparticles in suspensions (%DW) after centrifugation at 1500 × *g* for 15 min at 25 °C, either directly after nanoprecipitation or after 1 week storage at room temperature.

For all polymers, formation of aggregates during nanoprecipitation was enhanced by increasing polymer concentration in organic solvent as indicated by both the strong decrease of dry extract of suspensions after centrifugation and the higher particle diameters before centrifugation. The amount of submicronic particles in the suspensions was found to decrease with time and the aggregation process was accelerated upon increasing polymer concentration in the organic phase, i.e. the dry weight of nanoparticles. Indeed, after 1 week storage, of the dry weight of nanoparticles strongly decreased especially at high polymer concentrations in all cases except for nanoparticles prepared from 80% modified dextran at 5 g/L. This better stability may be due to the smaller particle size

Table 2
Average particle size and dry extract after centrifugation of nanoparticle suspensions obtained with different nanoprecipitation conditions.

DS (%)	Conc. (g/L)[a]	D_z (nm)[b,c]	Centrifuged after preparation		Centrifuged after 1 week	
			DW (%)[d]	D_z (nm)[c]	DW (%)[d]	D_z (nm)[c]
25[e]	5	206	52	169	13	233
	10	256	2	211	0.1	183
80[f]	5	86	96	95	96	107
	10	120	89	120	41	136
	20	155	62	139	10	151
150[f]	5	126	85	127	33	130
	10	203	29	179	7	181
	20	215	2	242	0.1	201

[a] Concentration of dextran ester in THF or THF/water solution.
[b] Mean diameter of nanoparticles measured after preparation and before centrifugation.
[c] Diameter values are averages of three successive measurements. Standard deviation is ±5 nm.
[d] Dry weight of nanoparticles remaining in suspension after centrifugation.
[e] Dissolved in THF/water mixture (90/10, v/v).
[f] Dissolved in THF.

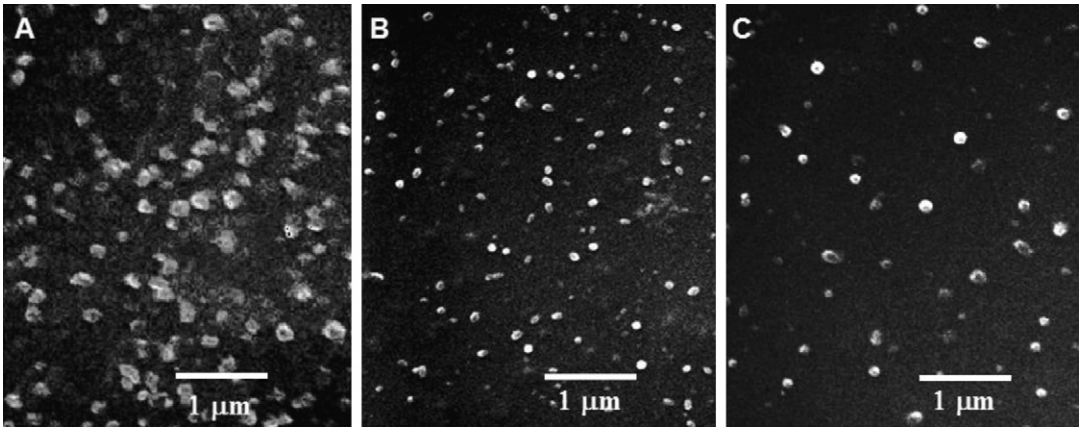

Fig. 2. SEM images of nanoparticles obtained from dextran derivatives with various DS: (a) DS = 25%, (b) DS = 80% and (c) DS = 150%. [Dextran ester] = 5 g/L; $V_{organic\ phase}$ = 5 mL.

obtained in this case as well as to the DS value of the polymer (see below). From these results, it can be concluded that polymer concentration plays an important role in nanoparticles formation upon solvent diffusion. The agglomeration of nanoparticles is favored when dry weight of nanoparticles increases. The higher the polymer concentration in THF, the higher the solid content of resulting nanoparticle suspensions is, thus increasing the probability of collision and aggregation of particles. At concentrations higher than 5 g/L individual particles are still formed but aggregation is statistically favored thus leading to the formation of higher amounts of aggregates. Analogous phenomena and interpretations have been reported and proposed (Galindo-Rodriguez, Allémann, Fessi, & Doelker, 2004; Gavory et al., 2011). The concentration of 5 g/L was thus chosen for further experiments in order to optimize both dry weight of nanoparticles and particle size.

3.3. Effect of DS on size and colloidal stability of nanoparticles

The effect of DS of dextran esters on initial nanoparticle diameter was investigated. Modification in DS gives rise to variations of different parameters such as polymer conformation or solution viscosity. As shown in Table 2, nanoparticle diameter increased from 86 to 126 nm when DS was increased from 80 to 150%. Similar result has been reported previously for nanoparticles prepared from dextran grafted with phenoxy group at different DS (Aumelas et al., 2007). Larger particle size was observed for the 25% modified dextran. This size increase may be due to less numerous interactions between hydrophobic groups of low modified dextrans leading to a loose hydrophobic core whereas numerous interactions of highly modified ones led to dense hydrophobic core. Indeed, in the case of dextran derivative with DS = 25%, the weight fraction of hydrophobically modified units is roughly 40%, which is much lower than for dextran derivatives with DS = 80 or 150% for which the weight fraction of hydrophobic units is about 89 and 100%, respectively (estimations based on DS values determined by ^{1}H NMR). Additionally, the solvent used for nanoprecipitation as well as polymer conformation could affect the size of nanoparticles (Legrand et al., 2007). We do not have detailed results about polymer conformation in acetone or THF. Additionally, as we showed in a previous paper, polymer conformation may also vary during solvent inter-diffusion, which probably also modifies the characteristics of resulting nanoparticles (Gavory et al., 2011). Nevertheless, we can consider that higher DS values favor more expanded conformations of macromolecules in THF. Indeed, polymers with DS equal to 25% are not soluble in THF. Hydroxyl groups are not solvated by THF thus the presence of modified repeat units favors contacts with solvent molecules and consequently expanded conformations.

Differences between particles obtained with the high and low modified dextrans evidenced by DLS were confirmed by SEM images (Fig. 2). Regular spheres with smooth nanoparticles surfaces were found when DS was above 80% whereas irregular particles were formed when using 25% modified dextran.

As a conclusion, the formation of dense and regular nanoparticles using dextran ester relies on a delicate balance between hydrophobic units producing the dense core and hydrophilic hydroxyl groups ensuring the formation of a hydrophilic superficial layer. It seems that DS = 80% is close to an optimum value but satisfactory colloidal stability is observed only for low dry weight of nanoparticles (5 g/L of dextran ester in THF). Thus, the addition of a low DS dextran ester in the aqueous phase was investigated as a way to improve colloidal stability independently of the DS of dextran ester in the core of nanoparticles.

3.4. Effect of polymeric surfactant on size and colloidal stability of nanoparticles

Further investigations were carried out to improve stability of nanoparticles by using polymeric surfactant in aqueous solution. Water-soluble modified dextrans have been reported as efficient polymeric stabilizers to improve stability of nanoparticles made of poly(lactic acid) (PLA) or modified dextran (Aumelas et al., 2007; Rouzes, Gref, Léonard, De Sousa Delgado, & Dellacherie, 2000; Rouzes et al., 2003). In this study, a water-soluble dextran derivative, with a DS of 15% was used as polymeric surfactant during nanoprecipitation in order to form a hydrophilic corona onto hydrophobic nanoparticles. As presented in Table 3, the difference in size of nanoparticles obtained with and without surfactant was not significant. This confirms that during nanoprecipitation the size of obtained particles is mainly controlled by thermodynamic

Table 3
Mean diameter of nanoparticles prepared by nanoprecipitation with and without 1 g/L of 15% modified dextran in the aqueous phase using 5 g/L of modified dextran in 5 mL of THF or THF/water.

Modified dextran (DS in %)	Water		[15% Dextran ester]$_{water}$ = 1 g/L	
	D_z (nm)[a]	PDI[b]	D_z (nm)[a]	PDI[b]
25	206	0.11	208	0.09
80	86	0.11	84	0.06
150	126	0.09	114	0.03

[a] Diameter values are averages of three successive measurements. Standard deviation is ±5 nm.
[b] Polydispersity index, for definition and significance, see Section 2.

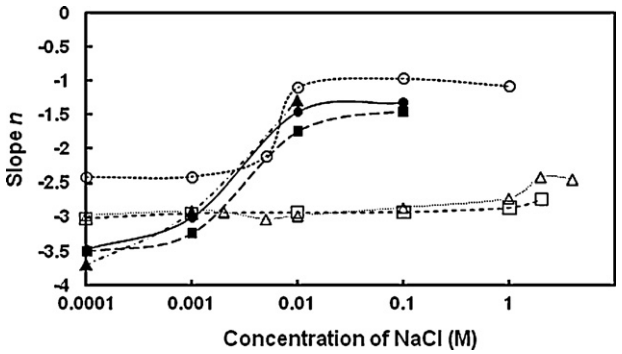

Fig. 3. n, $d(\log A)/d(\log \lambda)$, as a function of NaCl concentration for bare (closed symbols) and polymeric surfactant-covered (open symbols) particles obtained from dextran derivatives with DS of 25% (circles), 80% (squares) and 150% modified dextran (triangles). The polymeric stabilizer was a dextran ester with DS = 15%.

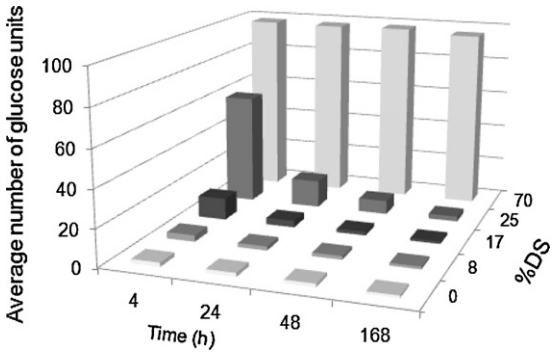

Fig. 5. Enzymatic degradation by dextranase of modified dextran with low DS as a function of time.

parameters like nature of solvent or polymer concentration but not by the use of a stabilizer.

The colloidal stability of nanoparticle suspensions was characterized at various concentrations of NaCl. The critical flocculation concentration was determined at the slope breaking of n as a function of NaCl concentration as presented in Fig. 3. It was found that nanoparticles produced without polymeric surfactant were no longer stable when NaCl concentration exceeded a critical value between 10^{-3} and 10^{-2} M. Particle flocculation may be caused by an increase of Van der Waals attractions between particles upon increasing of ionic strength through hydrophobic association between alkyl tails. In the presence of polymeric surfactant, nanoparticles prepared from 80 and 150% modified dextran were stable up to 1 M NaCl. These results underline the increase of colloidal stability of nanoparticles when using 15% modified dextran as stabilizer. It can be explained by steric stabilization, due to osmotic and elastic compression of the stabilizer layer at the surface of the particle. No improvement of colloidal stability by surfactant was observed for nanoparticles obtained from 25% modified dextran. A lower amount of adsorbed surfactant on their surface, due to their higher hydrophilicity (hydrophilic units represent about 60 wt% in the copolymer), may be responsible for the low colloidal stability of these nanoparticles (Gavory et al., 2011).

3.5. Enzymatic degradation of dextran esters

Two types of enzyme-catalyzed degradation of nanoparticles were investigated: hydrolysis of polysaccharide backbone in the presence of dextranase and hydrolysis of ester links between glucose units and hydrocarbon tails in the presence of porcine pancreatic lipase (Fig. 4).

3.5.1. Degradation by dextranase

Degradability of dextran derivatives by dextranase is influenced by the type of functional groups and DS of modified dextran (Aumelas et al., 2007; Mehvar, 2000). Degradation rate of dextran modified with ethylglycidyl ether, epoxyoctane or epoxydodecane decreased with increasing DS (Aumelas et al., 2007). The presence of dextranase in difference tissues such as kidney, colon, spleen and lung but not in blood makes dextran derivatives ideal candidate for targeted delivery of therapeutic agents (Frazier et al., 1997; Hovgaard & Brondsted, 1995; Kamath & Park, 1995).

In this work, degradation of dextran esters by dextranase was evaluated at various time of incubation (4, 24, 48 and 168 h). The cleavage of dextran by dextranase led to oligosaccharide reducing chain ends (Fig. 4) which were titrated by Sumner reagent. The number of glucose units in the final fragments could be calculated from these results. As shown in Fig. 5, native dextran was completely degraded in 4 h. Final products of native dextran degradation have been reported by other authors as isomaltose, dextrose or glucose and other oligosaccharides (Franssen, van Ooijen, de Boer, Maes, & Hennink, 1999; Sery & Hehre, 1956). For dextran esters we found that polymers with DS lower than 25% were completely degraded after 168 h. On the contrary, the degradation of highly modified dextran was much slower. The reason might be the insolubility in water of these dextran derivatives, unfavourable to enzymatic catalysis. Thus we investigated the possible hydrolysis of dextran esters in the presence of lipase. Hydrolysis of ester

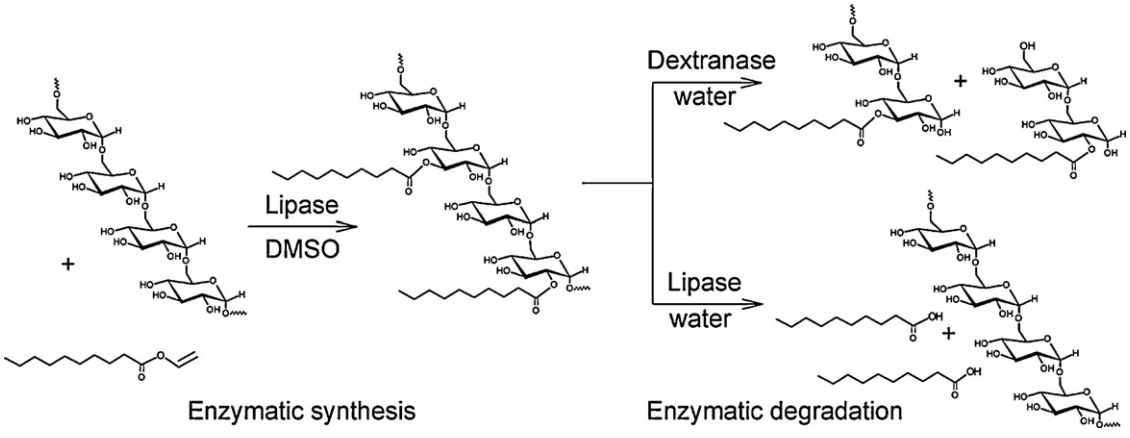

Fig. 4. Schematic representation of enzyme-catalyzed synthesis and degradations of dextran ester-based nanoparticles.

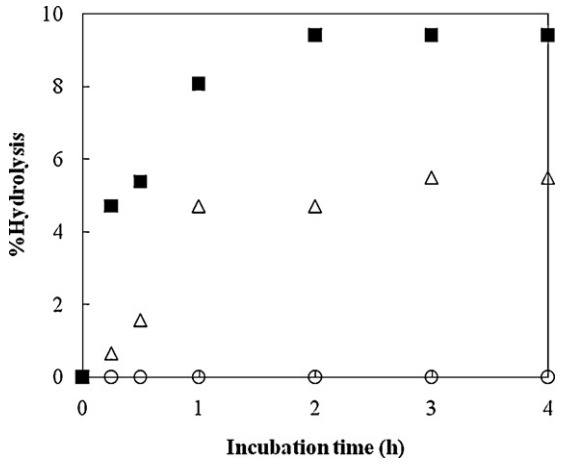

Fig. 6. Hydrolysis of ester bond of modified dextrans with DS of 21% (circles), 89% (squares) and 150% (triangles) by porcine pancreatic lipase as function of time.

bond would produce dextran esters with lower DS that could be further degraded by both lipase and dextranase.

3.5.2. Degradation by porcine pancreatic lipase

Pancreatic lipase is a primary enzyme that hydrolyzes ester bonds found in human digestive system. It has been used to evaluate degradability of biomaterial such as polyesters employed for biomedical applications (Peng et al., 2010). Nevertheless, to the best of our knowledge, the enzymatic degradation by lipase of dextran esters with DS as high as 89 and 150% has never been reported. In this study, the degradation of three modified dextrans with DS of 21, 89 and 150% in the presence of porcine pancreatic lipase was carried out. The cleavage of ester bonds between the glucose units of dextran backbone and carboxylate side chains was determined by measuring the amount of liberated fatty acid (Fig. 4). Fig. 6 shows the hydrolysis of modified dextrans after 4 h of incubation with pancreatic lipase at 37 °C. The degradation of dextran esters with DS of 89 and 150% increased with the time of incubation and reached the highest % hydrolysis (9.4 and 5.5%) after 2 and 3 h of incubation, respectively. Higher hydrophobic modification lowered the hydrolysis of modified dextran. No degradation could be detected for the 21% modified dextran probably because of the low initial amount of ester attached to dextran backbone. Since no detailed study about enzymatic hydrolysis of dextran esters is available in the literature, we were not able to compare our results to other. Only one paper reported the release of protein from modified dextran-based nanoparticles after degradation by lipase (Ge et al., 2011). Nevertheless, the hydrolytic activity of modified dextran by lipase has not been investigated. The low hydrolysis of modified dextran could be explained by the arrangement of molecule. In aqueous solution of hydrolysis by lipase, the arrangement of macromolecules with exposing dextran backbone to the surface of nanoparticles and hiding the hydrocarbon chains inside the core of nanoparticles could limit degradation by lipase due to low ester on the surface of nanoparticle.

The capacity of porcine pancreatic lipase to degrade ester bonds of highly modified dextran offers the possibility to use them to prepare nanoparticles for drug delivery systems. Indeed, combination of lipase and dextranase catalytic activities would lead to a complete degradation of nanoparticles. The overall kinetics of nanoparticle degradation may be adjusted by the DS of dextran ester depending on the required extent of lipase-catalyzed hydrolysis of ester bonds before dextranase hydrolysis of polysaccharide backbone could take place. Thus the ability of extending DS up to very high values may be an opportunity to modulate degradation kinetics of nanoparticles.

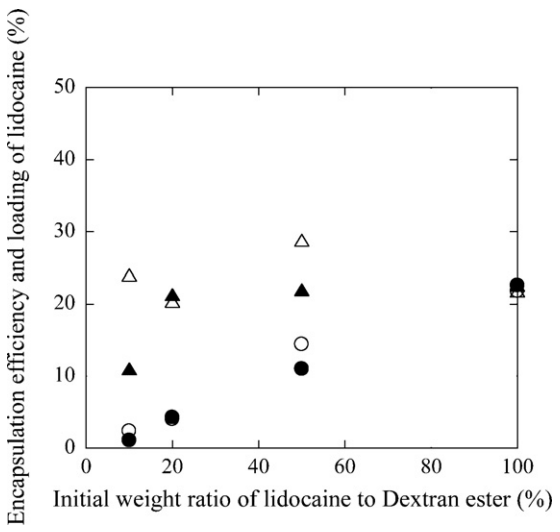

Fig. 7. Encapsulation efficiency (triangles, weight fraction of encapsulated molecules as compared to the feed) and loading (circles, weight fraction of encapsulated molecule in nanoparticles core) of lidocaine as a function of initial weight ratio of lidocaine to dextran ester. DS of dextran ester was 150%. Nanoprecipitation was carried out with (closed symbols) or without (open symbols) stabilizer (dextran ester with DS = 15%) in the aqueous phase. Results obtained with dextran esters having DS = 21 and 89% were very close to those shown on the graph.

3.6. Encapsulation of lidocaine

Encapsulation ability of modified dextran-based nanoparticles was examined by using lidocaine as a common hydrophobic drug. Lidocaine was added at various concentrations (0.5, 1, 2.5 and 5 g/L) to a solution of modified dextran (5 g/L). The amount of lidocaine loaded in nanoparticles was determined by indirect method (determination of free lidocaine remaining in supernatant after centrifugation). Significant amounts of lidocaine (lidocaine loadings between 11 and 29% (w/w)) were incorporated into nanoparticles obtained from dextran esters with different DS between 21 and 150%, both with and without stabilizer in the aqueous phase (Fig. 7). Encapsulation efficiency was defined as the ratio of the weight of encapsulated lidocaine to the initial weight of lidocaine in the feed). We observed that encapsulation efficiency did not vary with lidocaine loading and remained close to 18% in all conditions. These results were similar to those obtained for encapsulation of lidocaine in PLA using emulsion/solvent evaporation procedure (Görner et al., 1999). They showed that encapsulation efficiency was mainly influenced by the PLA concentration in the organic solvent and almost insensitive to lidocaine content. Within the range of conditions examined in this work, lidocaine loading varied continuously with the feed content without reaching a saturation limit (Fig. 7).

In any case, nanoparticle size increased with increasing concentration of lidocaine (Fig. 8). Nevertheless, this variation was limited to less than 40 nm for DS = 89 and 150% while it was considerable for DS = 21%. When 15% modified dextran was added in the aqueous phase as stabilizer, sizes of loaded nanoparticles slightly increased except for nanoparticles obtained from 21% modified dextran. In that case, sizes of loaded nanoparticles strongly increased with the concentration of lidocaine in the absence of surfactant. This fact may be attributed to the lose particle core obtained with DS = 21% which swells upon lidocaine loading, leading to particle aggregation during nanoprecipitation. For denser nanoparticles like those obtained with DS = 89 and 150%, particle aggregation is not increased in the presence of lidocaine. The use of a macromolecular stabilizer allows limiting this aggregation.

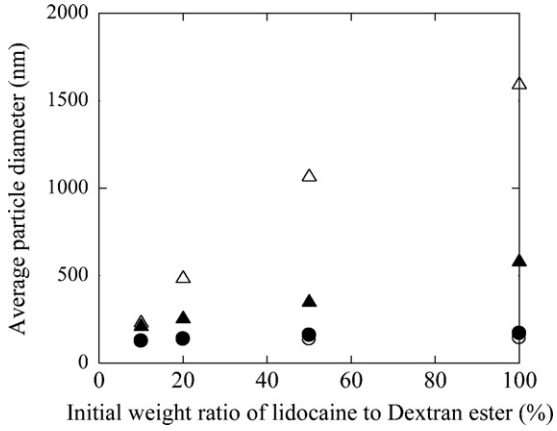

Fig. 8. Average particle diameter as a function of initial weight ratio of lidocaine to dextran ester. DS of dextran ester was 21% (triangles) and 150% (circles). Nanoprecipitation was carried out with (closed symbols) or without (open symbols) stabilizer (dextran ester with DS = 15%) in the aqueous phase.

4. Conclusion

Nanoparticles based on new biopolymers, namely dextran decanoate esters, were formulated by nanoprecipitation method. Nanoparticles could be obtained by using two derivatives: a low modified water soluble one and a high modified water insoluble one. The size and stability of nanoparticles were found to strongly depend on dextran concentration and degree of substitution. Better colloidal stability vs. ionic strength was obtained when a low modified dextran derivative with DS = 15%, was added in water during nanoprecipitation. The combination of dextranase and pancreatic lipase to degrade modified dextrans with DS between 20 and 150% offers the possibility to use these polymers for biomedical applications. Significant amounts of lidocaine could be encapsulated into nanoparticles. The degree of substitution of dextran esters was shown to be a relevant parameter for modifying both colloidal characteristics and degradation kinetics of nanoparticles. These results pave the way for further studies to formulate dextran based biodegradable nanoparticles for drug delivery applications.

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