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Macromolecular surfactants synthesized by lipase-catalyzed transesterification of dextran with vinyl decanoate

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ABSTRACT

Dextran, a neutral bacterial polysaccharide consisting in glucose units linked in α-1,6 was modified by covalent attachment of linear aliphatic hydrocarbon groups via the formation of ester links. Transesterification reaction between dextran and vinyl dodecanoate was carried out in a polar organic solvent (dimethylsulfoxide) and catalyzed by lipase. Modified dextrans were fractionated using solvents of increasing polarity (ethyl acetate, methanol and water). Except for very low amounts of enzyme, modified polymers covered a range of degrees of substitution (ca. from 10 to 150%, defined as the molar ratio of formed ester bonds to available glucose units) with distribution depending on the initial amounts of reactants and lipase. Soluble fractions recovered at each step exhibited increasing degrees of substitution when decreasing solvent polarity (from water to methanol and ethyl acetate). In comparison, modified dextrans prepared by uncatalyzed transesterification (even over much longer times) exhibited degrees of substitution lower than 15% covering narrower ranges. The adsorption of modified dextrans at oil/water and air/water interface was examined by surface pressure and interfacial tension measurements for degrees of substitution varying from 10 to 150%.

1. Introduction

Dextran is a bacterial polysaccharide that has many advantages for preparing numerous materials in view of biomedical applications: nanoparticles or hydrogels for instance. Indeed, it is a biocompatible polymer and can be degraded by an enzyme, dextranase, which is present in human body (Frazier et al., 1997; Hovgaard & Brondsted, 1995; Kamath & Park, 1995). Dextran is a mainly linear polysaccharide formed by neutral repeat units containing hydroxyl groups (Nordmeier, 1993). Dextran samples with molar masses lower than 100,000 g/mol are the most frequently used. Hydroxyl groups of repeat units can be used for the covalent attachment of various organic functional groups onto dextran chains. Such functional groups may have several interests like providing biological specificities to dextran macromolecules, allowing spectrophotometric detection or enabling macromolecular auto-organization in the biological medium for applications like encapsulation or solubilization of drugs. In particular, dextran has been chemically modified through the covalent attachment of hydrophobic functional groups (linear hydrocarbon chains or aromatic rings). The amount of attached hydrocarbon tails is usually expressed by defining the substitution degree as the molar ratio of attached hydrophobic groups to overall glucose repeat units contained in dextran chains. When high enough degrees of substitution are reached (according to the nature of attached groups it may vary from 15% to 100%) polymers become water insoluble and are able to self-organize at the submicrometer scale in water (Aumelas, Serrero, Durand, Dellacherie, & Léonard, 2007; Hornig & Heinze, 2007). One concern about the resulting nanoparticles is their biodegradability since, for instance, it has been shown that enzymatic degradation of dextran is significantly slowed down when the polysaccharide is chemically modified by hydrophobic groups (Aumelas et al., 2007). Thus the use of ester links may be promising since their hydrolysis in biological medium may strongly favor further degradation of dextran backbone even at high degrees of substitution. Another question to be raised is the possible activation of immune system by dextran-based nanoparticles. The stealthiness of nanoparticles is strongly influenced by their surface characteristics (Vonarbourg, Passirani, Saulnier, & Benoit, 2006). Protein adsorption as well as complement activation by dextran-covered nanoparticles depends on chain configuration (loops or brush), molar mass as well as on the characteristics

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of the proteins themselves (Chauvierre et al., 2010; Lemarchand et al., 2006; Osterberg et al., 1995; Passirani, Barratt, Devissaguet, & Labarre, 1998; Vauthier, Persson, Lindner, & Cabane, 2011). Brush-like surface coverage by dextran chains has been shown to suppress complement activation (Bertholon, Vauthier, & Labarre, 2006). Thus, because of their conformational diversity, dextran-based amphiphilic polymers are interesting materials to produce nanoparticles with convenient biological interactions depending on the targeted behavior (slow/fast release or degradation, targeted delivery, etc.).

Enzymatic catalysis reduces the use of toxic reactants, allows proceeding in soft conditions (low temperature and pressure) and may induce a specificity of chemical modification. These aspects may be of interest for the synthesis of macromolecules with targeted biomedical applications. While previous studies demonstrated the efficiency of applying enzymatic catalysis to the synthesis of modified polysaccharides by transesterification, most of the reported studies aimed at grafting a limited number of cross-linking groups in order to prepare covalent dextran hydrogels (Carvalho, Gonçalves, Gil, & Gama, 2007; Ferreira, Carvalho, Gil, & Dordick, 2002; Ferreira, Gil, Cabrita, & Dordick, 2005; Ferreira, Gil, & Dordick, 2002; Ge, Lu, Wang, & Liu, 2009; Yang & Wang, 2004). There was no attempt to obtain highly modified polysaccharides by that way (Table 1). We followed a different approach through the preparation of water insoluble dextran esters carrying long hydrocarbon chains which may induce the formation of polymeric nanoparticles in an aqueous environment. Such colloids could be used for encapsulation and release of hydrophobic drugs. In addition, variation in the degree of substitution offers a way to adjust the polarity of the inner core of nanoparticles.

We showed that lipase-catalyzed transesterification between dextran and vinyl esters led to the synthesis of dextran fatty esters with average degrees of substitution as high as 96%, depending on the feed composition and on the reaction procedure. The effect of chemical structure of hydrocarbon chain of vinyl esters on the regioselectivity of covalent attachment was also detailed (Kaewprapan, Tuchinda, Marie, Durand, & Inprakhon, 2007; Kaewprapan et al., 2011).

In this work we further characterized the chemical structure of dextran fatty esters obtained by enzyme-catalyzed transesterification and compared these polymers to those obtained by a non catalyzed reaction. In particular, we examined the variability of substitution degree within modified dextran samples using solvent fractionation. Finally, the adsorption of low and highly modified dextrans at oil/water and air/water interface was characterized.

2. Experimental

2.1. Materials

Dextran T40 (from *Leuconostoc mesenteriodes*), with weight average molecular weight ($\overline{M_w}$) equal to 40,000 g/mol (according to size exclusion chromatography measurements) was purchased from Pharmacosmos (Holbaek, Denmark). DMSO (CROMASOV®), vinyl decanoate, 18-crown-6 ether and dodecane were purchased from Sigma–Aldrich (Buchs, Switzerland). Dialysis membrane with MWCO 6000–8000 was purchased from Spectrum Laboratories Inc. (CA, USA). Lipase AY, a commercial lipase (EC 3.1.1.3) from *Candida rugosa* (Lot: LAYE0151016), was purchased from Amano Enzyme Co. (Nagoya, Japan). Ethyl acetate, methanol, tetrahydrofuran were HPLC grade and purchased from Merck. All other chemicals and solvents were of analytical grade and used without further purification.

2.2. Transesterification of dextran with vinyl decanoate

2.2.1. Preparation of lipase co-lyophilized with 18-crown-6

A typical procedure can be described as follows. One gram of lipase AY was dissolved in 20 mL of 20 mM phosphate buffer pH 7.5 in the presence of 18-crown-6 at 11, 23, 46 or 92 μmol. The enzymatic mixture was stirred at room temperature for 1 h. Then, the solution was flash-frozen in liquid nitrogen followed by lyophilization with freeze dryer (Labcongo, USA) for 48 h. The water content of lyophilized powder was determined by moisture analyzer (Sartorius MA 30, Medtel, Australia). The lyophilized pH-imprinted enzyme contained approximately 15% of water. The obtained lyophilized pH-imprinted enzyme contained 25, 50, 100 and 200 mol equivalent of 18-crown-6 respectively.

2.2.2. Lipase-catalyzed transesterification of dextran with vinyl ester

A typical mixture used for transesterification contained 0.1 M of dextran T40 (expressed in moles of hydroxyl groups of the polymer, DexT40, 3 hydroxyl groups per repeat unit) and 0.4 M of vinyl decanoate ($[OH]_0/[ester]_0 = 1/4$) in 5 ml of DMSO, with DexT40 being dissolved in 5 ml DMSO prior to its mixing with vinyl decanoate. Water content of reaction systems was determined by Karl Fischer titration (C30 Compact Karl Fischer Coulometer, Mettler Toledo, Germany). The temperature of reaction mixture was raised to 50 °C and kept constant for 5 min before addition of 26 mg of pH-imprinted lipase AY (prepared as described previously) (Kaewprapan et al., 2007). The enzymatic solution was left under stirring at 50 °C for 32 h. The reaction was stopped and the DMSO solution was dialyzed against ethanol water mixture (85:15%, v/v) for 2 days followed by 60:40 (%, v/v) mixture for 2 days and then with distilled water for 2 days. Finally, the aqueous solution was lyophilized for 48 h. Control experiments without enzyme were carried out following the same procedure.

2.2.3. Characterization of modified dextrans

The degree of substitution (DS) of polymer samples defined as the molar ratio of attached ester groups to glucopyranoside units of polysaccharide (%) was determined by ^{1}H NMR (300 MHz) after dissolving polymers in DMSO-d_6.

The signal of residual methylic protons of DMSO, used as reference signal, was set at δ 2.47 ppm. ^{1}H NMR (δ, DMSO-d_6, ppm) peaks assigned were: 4.91 (O—H4) 4.86 (O—H3), 4.66 (H1), 4.51 (O—H2), 3.73 (H6), 3.61 (H5), 3.49 (H6), 3.41 (H3), 3.2 (H2) 3.15 (H4), 2.29 (—CH$_2$—C=O), 1.25 (—CH$_2$—CH$_2$—C=O), 1.23 (—CH$_2$—, 8H), 0.84 (—CH$_3$).

For the calculation of DS, the signal at 0.84 ppm corresponding to 3 methylic protons of the hydrocarbon chain of decanoate ($I_1 = 3$H) and the signal (I_2) of the proton of anomeric carbon of glucopyranoside unit of DexT40, were used (Eq. (1)).

$$\mathrm{DS}\,(\%) = \left[\frac{I_1/3}{(I_1/3) + ((I_2 - I_1)/4)}\right] \times 100 \quad (1)$$

2.3. Fractionation of dextran fatty esters

Modified dextrans with various DS were successively fractioned by ethyl acetate, methanol and water. In the first step, 1 g of modified dextran was suspended in 200 ml of ethyl acetate with vigorous stirring for 1 h. Then, the suspension was filtrated through borosilicate filter No. 4. Supernatant was evaporated under vacuum by rotary evaporator. Precipitate and solid residue from supernatant was left under vacuum for 24 h. Dry weights of soluble and non-soluble polymer in ethyl acetate were measured. DS of both parts were determined by ^{1}H NMR analysis. The non-soluble polymers were further fractioned in methanol and in water with the same

Table 1
Enzyme-catalyzed transesterification with polysaccharides reported in literature.

Polysaccharide[a]	Ester[a]	Enzymes; solvent	DS	Reference
CMC	VAc	*A. Niger* lipase A12; Phosphate buffer	Up to 2%	Yang and Wang (2004)
Dextran	VD	Lipase AY; DMSO	Up to 96%	Kaewprapan et al. (2007, 2011)
Dextran	VD	Lipase nanogel (*C. rugosa* with AAM network); DMSO	Up to 23%	Ge et al. (2009)
Dextran	VA	Proleather FG-F, lipase AY; DMSO	Up to 37%	Ferreira, Carvalho, et al. (2002) and Ferreira, Gil, et al. (2002)
Dextran	DVA	Proleather FG-F, lipases (*C. rugosa*, *P. cepacia*); DMSO	Up to 33%	Ferreira et al. (2005)
Dextrin	VA	Proleather FG-F; DMSO	Up to 67%	Carvalho et al. (2007)
Inulin	VA	Proleather; DMF	Up to 37%	Ferreira, Carvalho, et al. (2002) and Ferreira, Gil, et al. (2002)

[a] CMC, carboxymethyl cellulose sodium salt; DVA, divinyladipate; VA, vinyl acrylate; VAc, vinyl acetate; VD, vinyl decanoate.

procedure. Finally, the fractionation in water was done with the same proportion of polymer and solvent as described above for 1 h. The suspension was centrifuged at 3000 rpm for 15 min. Supernatant was separated and washed 3 times with Milli Q water. Supernatant and precipitate were flash-frozen in liquid nitrogen and dried by using freeze dryer for 48 h. Dry weights and DS were determined as described above.

2.4. Interfacial tension measurements

Interfacial tension measurements were carried out at 25 °C using a K8 surface tensiometer (Krüss, Germany). Only water-soluble dextran derivatives were used for those experiments. Solutions were prepared with ultrapure water (Milli Q water purification system, Millipore) and allowed to stand overnight at 4 °C before measurement. Interfacial tension was determined with a platinum ring at water/oil solution interface (Lecomte de Noüy method). The Harkins/Jordan correction was applied. Experimental errors were in the 0.2 mN/m range.

2.5. Langmuir–Blodgett films experiments

Monolayers of water-insoluble dextran derivatives were formed by spreading of polymer solutions (in THF or ethyl acetate) with a microsyringe on pure water as subphase. A polymer concentration of 1 g/L in organic solvent was prepared 24 h before measurement. The films were prepared in a trough having a moving barrier. Compression was initiated 10 min after spreading the amphiphilic molecules, which allowed a polymer layer to be formed on pure water. The π–A isotherms were measured at various barrier speeds (1, 2, 5 and 10 mm/min) by a computer-controlled KSV-1100 film balance system (KSV Instruments, Helsinki, Finland) and the surface pressure by a platinum Wilhelmy plate. The maximum surface area was 242 cm^2 and the subphase was maintained at a temperature of 25 °C.

2.6. Capillary viscometry

Modified dextrans with different DS were dissolved in DMSO at a concentration of 5 g/L. Solutions were prepared for 24 h before the measurement. Reduced viscosity measurements were made using a S5 Lauda injection pump, Lauda E200 Water Bath (set to 30 °C), Lauda Processor Viscosity System and a Schott 53110 Capillary Viscometer Tube, 0.53 mm diameter. Reduced viscosity was calculated using Eq. (2):

$$\eta_{red} = \frac{t - t_0}{t_0 C} \quad (2)$$

In Eq. (2), η_{red} is the reduced viscosity (L/g), t and t_0 are the flow time of polymer solution and pure DMSO, respectively, C is polymer concentration (in g/L).

3. Results and discussion

3.1. Lipase-catalyzed modification of dextran

3.1.1. Effect of reaction conditions on degree of substitution (DS)

The degree of substitution (DS) was controlled by changing reaction conditions: amounts of reactants (vinyl decanoate and dextran), amount and pre-treatment of enzyme, way of enzyme addition (Table 2). When no enzyme was added, DS did not exceed 12% even after 240 h of reaction (lines 1 and 2 in Table 2). On the contrary, in the presence of enzyme, it was possible to reach 77% substitution after 48 h. A detailed discussion of the decrease of lipase activity in DMSO as well as how the combination of pH-adjustment of enzyme and crown ether addition significantly improved dextran modification as demonstrated previously is available in a recent paper (Kaewprapan et al., 2011). Addition of enzyme in two steps led to a significant increase of DS. In particular, addition of enzyme in two steps allowed a significant reduction of the required amount of catalyst while keeping high DS values and similar reaction times (compare lines 8 and 9, Table 2). Previously we showed that with three steps 96% substitution could be reached (Kaewprapan et al., 2011).

We compared the kinetics of lipase-catalyzed modification starting either from native dextran or from a modified dextran with DS = 25% (Fig. 1). Both polymers were modified by transesterification. Nevertheless, similar DS values were attained after 6 h of reaction and remained close to each other up to 10 h of reaction. Thus starting from an already modified dextran did not allow reaching significantly higher DS. Enzyme-catalyzed transesterification seems slower with modified dextrans than with the native polysaccharide. We will comment further that point by considering in more detail the distribution of DS within the product of reaction.

Table 2
Experimental conditions used for enzyme-catalyzed dextran modification and degree of substitution (DS) of modified dextran determined by ^{1}H NMR.

Sample no.	pH-adjusted lipase (mg)	DexT40:VD[b]	Time (h)	DS (%)
1	–[a]	1:2	218	11
2		1:4	240	12
3			0.5	7
4	25[c]	1:4	2	18
5			24	35
6			48	31
7	25[c]	1:2	4	27
8	133[c]	1:4	32	63
9	25 (×2)[c,d]	1:4	48	77

[a] Control experiment.
[b] Molar ratio of dextran repeat units to vinyl decanoate.
[c] Lipase was pre-treated with crown ether (see Section 2).
[d] Lipase was added in two steps (see Section 2).

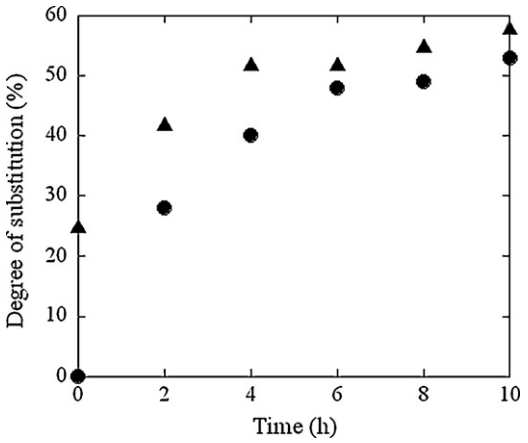

Fig. 1. Variation of degree of substitution as a function of reaction time for lipase-catalyzed transesterification of native dextran (●) and already modified dextran with DS = 25% (▲). For reaction conditions see Section 2.

3.1.2. Fractionation of reaction products

All samples of recovered dextran esters were fractionated using a series of three solvents of increasing polarity: ethyl acetate, methanol and water (see Section 2 for detailed procedure). For most of recovered fractions, DS was determined by ^{1}H NMR. The consistency of DS values with the weights of recovered fractions was checked by comparing the experimental DS value to the one calculated on the basis of fractionation results (Table 3). All polymer samples were readily soluble in dimethylsulfoxide.

Dextran esters obtained after enzyme-catalyzed transesterification cover a range of DS values between 8 and 150%. Only samples obtained either without enzyme or with enzyme but after reaction times lower than 4 h do not cover such a wide interval. These results demonstrate that, even if the DS of the product recovered after reaction is 60%, the polymer sample contains macromolecules with DS up to 150%. Such DS values are difficult to obtain by usual chemical modification. For all polymers obtained after at least 24 h reaction in the presence of enzyme, DS values of given fractions fall in the same range: 113–156% for fraction no. 1 soluble in ethyl acetate, 81–89% (except for sample 6) for fraction no. 2 soluble in methanol, 22–29% for fraction no. 3 insoluble in water and 10–19% for fraction no. 4 soluble in water.

These results show that polymers obtained after enzyme-catalyzed transesterification contain modified dextran molecules which cover a wide range of DS that may be associated to a range of polarity. A semi-quantitative interpretation of these results can be proposed on the basis of Hansen solubility parameters. Only a few papers dealt with the calculation of Hansen solubility parameter of dextran and its various components (dispersion, polar and hydrogen bonding, Table 4) (Antoniou & Alexandridis, 2010; Antoniou, Buitrago, Tsianou, & Alexandridis, 2010; Antoniou, Themistou, Sarkar, Tsianou, & Alexandridis, 2010; Antoniou, Tsianou, & Alexandridis, 2005; Güner, 2004; Icoz & Kokini, 2007). To the best of our knowledge, no application of this approach to dextran esters has been reported yet. Although all published values are reported to result from group contribution method, it is clear that there are significant differences. The exact reason for this spreading of calculated data is unclear. Using group contribution method from Van Krevelen and Hoftyzer, we calculated Hansen solubility parameters of dextran fatty esters having DS equal to 100, 200 and 300%, considering that all repeat units were uniformly substituted by 1, 2 or 3 fatty acid chains (Table 5). A qualitative comparison of polymer and solvent characteristics can be done using the diagram proposed by Bagley (δ_H in ordinate and $\delta_V = (\delta_D{}^2 + \delta_P{}^2)^{1/2}$ in abscissa, Fig. 2). We focused on 4 solvents: water, dimethylsulfoxide (DMSO), methanol and ethyl acetate. In this diagram, the representative point of modified polysaccharide is displaced from the vicinity of water (for DS = 0%) to that of ethyl acetate (for DS = 200 and 300%) upon hydrophobic substitution. The point representing methanol is located between that of native dextran and that of DS = 100%. Finally, the point of DMSO is close to those of dextran esters. These results are qualitatively consistent with the DS of the four fractions contained in most of modified dextrans (see above). The diagram shows that the main effect explaining the variation of solubility comes from the reduction of hydrogen bonding contribution by substituting hydroxyl groups by fatty esters. Another experimental illustration of that result was obtained when comparing the solubility of dextran derivatives as a function of DS in tetrahydrofuran (THF) and

Table 3
Fractionation of modified dextrans obtained after enzyme-catalyzed transesterification with vinyl decanoate. Fraction no. 1, soluble in ethyl acetate; fraction no. 2, soluble in methanol; fraction no. 3, insoluble in water; fraction no. 4, soluble in water. For sample numbers see Table 2.

Sample no.	Fraction no. 1		Fraction no. 2		Fraction no. 3		Fraction no. 4		DS_{exp} (%)	DS_{calc} (%)
	Weight fraction (%)	DS (%)	Weight fraction (%)	DS (%)	Weight fraction (%)	DS (%)	Weight fraction (%)	DS (%)		
1	0.6	n.d.	0.6	n.d.	0.0	–	88.1	10	11	10
2	0.3	n.d.	0.2	n.d.	0.7	12	86.8	11	12	11
3	0.2	n.d.	7.4	37	0.0	–	87.8	3	7	5
4	4.8	76	21.9	70	1.2	28	49.6	6	18	23
5	20.1	154	2.5	83	62.0	22	6.3	16	35	39
6	18.2	150	2.6	49	70.9	23	2.4	19	31	37
7	7.0	113	19.0	80	6.8	46	51.4	8	27	28
8	43.3	150	5.2	81	26.2	25	10.4	17	63	71
9	42.3	156	9.3	89	24.9	29	11.7	11	77	73

Table 4
Hansen solubility parameter of dextran (δ) and its dispersion (δ_D), polar (δ_P) and hydrogen bonding (δ_H) contributions.

δ ($J^{1/2}/cm^{3/2}$)	δ_D ($J^{1/2}/cm^{3/2}$)	δ_P ($J^{1/2}/cm^{3/2}$)	δ_H ($J^{1/2}/cm^{3/2}$)	Calculation method	Reference
40.4	–	–	–	Van Krevelen and Hoftyzer	Icoz and Kokini (2007)
46.3	–	–	–	Hoy	Icoz and Kokini (2007)
38.6	24.3	19.9	22.5	Not detailed	Antoniou et al. (2005), Antoniou, Buitrago, et al. (2010) and Antoniou, Themistou, et al. (2010)
31.4	13.2	15.0	24.2	Van Krevelen and Hoftyzer	Güner (2004)
30.3	13.3	18.0	20.4	Hoy	Güner (2004)

Table 5
Hansen solubility parameter of dextran and dextran fatty esters calculated by group contribution method from Van Krevelen and Hoftyzer.

Dextran fatty ester	δ ($J^{1/2}/cm^{3/2}$)	δ_D ($J^{1/2}/cm^{3/2}$)	δ_P ($J^{1/2}/cm^{3/2}$)	δ_H ($J^{1/2}/cm^{3/2}$)
DS = 0%	46.3	26.0	17.9	33.8
DS = 100%	24.8	18.9	4.6	15.3
DS = 200%	20.9	18.0	2.6	10.1
DS = 300%	18.9	17.5	1.8	6.9

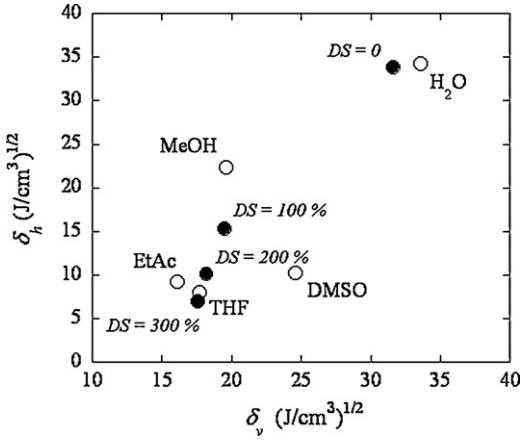

Fig. 2. Representative points of modified dextrans (●) and various solvents (○) in the δ_V–δ_H diagram (DS values and solvents are indicated on the graph).

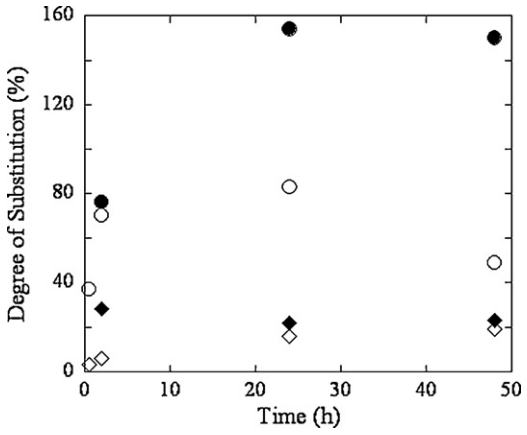

Fig. 3. Variation of degree of substitution of various fractions extracted from transesterification products as a function of reaction time: fraction no. 1 (●), fraction no. 2 (○), fraction no. 3 (♦) and fraction no. 4 (◊). For reaction conditions see Table 2.

in THF:water (90:10, v:v) mixture (Table 6). The addition of 10 wt% of water strongly modified the solubility characteristics of dextran derivatives favoring dissolution of low-modified polymers while restraining the solubility of highly modified derivatives.

3.1.3. Main characteristics of enzyme-catalyzed transesterification

Enzyme-catalyzed transesterification of dextran was monitored by characterizing the relative amounts of fractions 1–4 as well as their DS values over 48 h of reaction (Figs. 3 and 4). Over the whole reaction time, the DS value of water soluble fraction (no. 4) continuously increased from 3 to 19%. Over the same time, the DS value of polymer fraction soluble in ethyl acetate reached finally 150% after 24 h and did not increase further even after 48 h. On the contrary, the DS of water-insoluble fraction (no. 3) remained in a narrow range (22–28%). Finally, the DS of the methanol-soluble fraction (no. 2) reached a maximum (83%) after 24 h of reaction. This first series of results shows that dextran modification cannot exceed a maximum extent even after 48 h of reaction. This maximum extent of modification corresponded to a DS value of 150%, in the investigated range of reaction conditions. Thus, it seems that a maximum of two grafted hydrocarbon chain per glucose unit is to be found within dextran chains through enzyme-catalyzed transesterification. In addition, during the reaction, low modified chains were progressively converted into more modified ones, as illustrated by the continuous increase of DS of the water-soluble fraction, up to the limit of 20% (Fig. 3). Turning to the amounts of fractions 1–4 contained in the modified polymer, as a function of reaction time, we found consistent trends. Fraction no. 4 (water soluble polymers) decreased monotonically over the reaction time while fraction no. 3 underwent a strictly reverse variation with a continuous increase from 0 to 71 wt% after 48 h of reaction. Fraction no. 4 (highly modified polymers) increased from 0 to 18 wt% over the same period of time. Thus the final product of enzyme-catalyzed transesterification contained essentially a low-modified fraction (71 wt%) with a DS equal to 23% and a highly modified fraction (18 wt%) with a DS equal to 150%. Decreasing the amount of vinyl ester in the feed did not induce any significant variation as compared to previous results. Increasing the amount of lipase in the feed or adding a new load of lipase during the reaction increased significantly the fraction of highly modified polymers (up to 43 wt% as compared to 18 wt%)

Table 6
Solubility of dextran derivatives in THF and THF:water (90:10, v:v) mixture. The experiences were carried out with feed compositions equivalent to 1 g/L polymer solutions.

DS (%)	THF[a]	THF:water[a] (90:10, v:v)
150	*s*	*p s* (87%)
113	*s*	*p s* (80%)
80	*s*	*p s* (77%)
22	*i*	*s*
17	*i*	*i*
8	*i*	*i*

[a] *i*, insoluble; *p s*, partly soluble (with weight percent of solubilized material in parentheses); *s*, fully soluble.

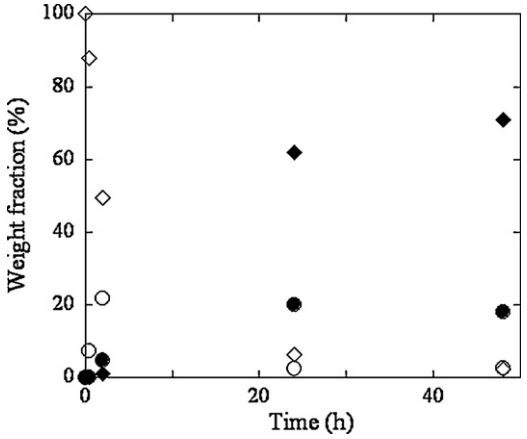

Fig. 4. Variation of weight percent of various fractions extracted from transesterification products as a function of reaction time: fraction no. 1 (●), fraction no. 2 (○), fraction no. 3 (♦), fraction no. 4 (◊). For reaction conditions see Table 2.

Table 7
Intrinsic viscosity of modified dextrans in DMSO at 25 °C.

Polymer	$[\eta]$ (mL/g)[a]
Native dextran	25.1
15%	22.6
21%	24.7
89%	7.4
150%	4.7

[a] Estimated by one-point methods (see text) from one measurement at 5 g/L.

while the fraction of low modified polymers was decreased down to 37 wt%. Nevertheless, even with higher amounts of enzyme in the reaction medium (either initially or by the addition of fresh enzyme during the reaction) the maximum value of DS was still 150% in the highly modified fraction. These results were consistent with the idea of a maximum extent of modification obtained by enzyme-catalyzed reaction. On the contrary, we have no indication that the amounts of the different populations of macromolecules attained a stationary value. Increasing reaction time may lead to a modification toward highly modified fraction.

3.1.4. Viscometric study

Reduced viscosity of dilute solutions of modified dextrans in DMSO at 25 °C was measured (Table 7) and the intrinsic viscosity of the polymers was estimated using the one point methods proposed by Solomon and Gottesman (1969) and Deb and Chatterjee (1969) (both giving very similar values). According to the supplier, the number-average molar mass of native dextran should be around 20,000 g/mol. Using the Mark–Houwink relation established by Catiker and Güner (1998), we can calculate a value of 20.6 mL/g for native dextran. This estimated value seems reasonably close to the experimental value of 25.1 mL/g.

From the results, it is obvious that highly modified dextrans exhibit a much lower intrinsic viscosity than native dextran and low modified derivatives. Even if fractionation of polymer samples may have induced variations of molar mass between the four separated fractions, this would not account for the 4-fold decrease observed when DS changes from 15% to 150%. Thus this contraction of dextran macromolecules may be attributed to the attachment of hydrocarbon tails along polysaccharide backbone which decrease solvent quality especially in the case of a polar solvent like DMSO. These trends are consistent with the previous discussion about fractionation results and the role of hydrogen bonds.

From these results, coil contraction upon attachment of hydrocarbon tails may be one reason for the limitation of modification extent of dextran molecules in DMSO. Indeed, several papers dealing with enzyme-catalyzed sugar esterification showed that di- or triesters can be formed (Arcos, Hill, & Otero, 2001; Ferrer, Cruces, Bernabé, Ballesteros, & Plou, 1999; Park, Jeon, & Yang, 1999; Shi, Li, & Chu, 2011; Soedjak & Spradlin, 1994). With polysaccharides, the covalent links between sugar repeat units create supplementary steric hindrance which may limit the formation of multi-substituted units. This steric hindrance may be further increased by contraction of polymer coil because of lower solvent quality for modified dextran chains.

3.1.5. Comparison with uncatalyzed transesterification

Uncatalyzed transesterification was carried out in the same conditions as before (Table 2). Even by extending reaction time up to 240 h, it was not possible to obtain DS values higher than 12%. In addition, fractionation did not allow extracting significant amounts of highly modified chains (Table 3). When comparing to samples obtained after 30 min or 2 h in the presence of enzyme (these samples have DS values below and above 12%), it appears that without enzyme highly modified chains cannot form, even after very long times.

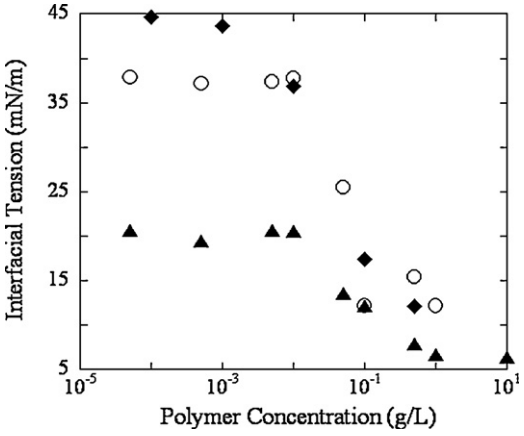

Fig. 5. Interfacial tension at 25 °C between various oil and water as a function of modified dextran (DS = 12%) concentration in water. Oils: Mygliol 810® (▲), silicon oil (○) and dodecane (♦).

3.2. Adsorption of modified dextrans at air/water and oil/water interfaces

The surfactant properties of modified dextrans were characterized by separating water soluble derivatives and water insoluble ones.

Interfacial tension was measured between aqueous polymer solutions and various oils, using a dextran derivative with DS = 12% (Fig. 5). Polymer concentration was varied between 5×10^{-4} and 10 g/L. Three oils differing by polarity were used: dodecane, Mygliol 810® and silicon oil. In all cases, a significant lowering of interfacial tension is observed within the concentration range 10^{-2}–1 g/L. These data show that dextran derivatives with DS lower than 20% may be used as stabilizers for dispersions of hydrophobic particles in aqueous medium. Their adsorption at oil/water interface gives rise to the formation of a dense layer of hydrophilic loops providing steric repulsions between particle surfaces.

In the case of dextran derivatives having DS higher than 20%, adsorption at air/water interface was characterized by compression isotherms of Langmuir–Blodgett films.

Compression isotherms of monolayers obtained with 3 dextran derivatives deposited from THF (DS = 80 and 150%) or THF:water mixture (90/10, v:v) (DS = 20%) show raising curves with narrow or no plateau regions (Fig. 6). Because of high density of hydrocarbon tails, interactions with aqueous subphase are reduced and macromolecules are present in the form of folded conformations at interface.

In the case of a highly modified dextran with DS = 150% compression isotherm was the same when the monolayer was deposited from THF or from ethyl acetate (Fig. 7). This was consistent with the high density of hydrocarbon tails grafted along the polysaccharide backbone which strongly limited interactions with the aqueous subphase, whatever the solvent polarity (either miscible with water or not). A very different result was observed with a less modified dextran (DS = 113%, Fig. 8). When ethyl acetate was used, the shape of compression isotherm was similar to that of DS = 150%. On the contrary, when THF was used, compression isotherm exhibited a plateau region indicating much more expanded macromolecules. It seems that this polymer covers a range of DS values for which THF is particularly favorable for chain extension which strongly modified chain conformation at air/water interface, promoting interactions with aqueous subphase. A more detailed study would be required

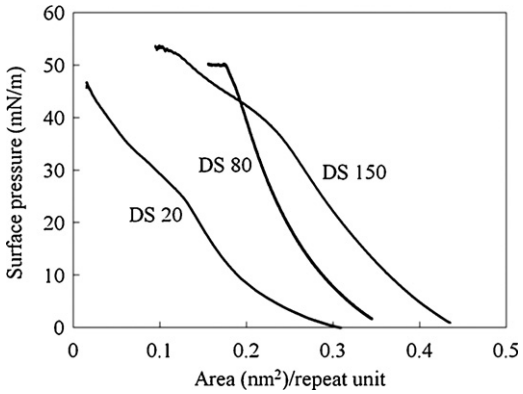

Fig. 6. Surface pressure isotherms obtained with dextran derivatives having degrees of substitution equal to 20, 80 and 150% (as indicated on the graph) after deposition from THF solution for (DS = 80 and 150%) or THF:water (90:10) solution (for DS = 20%) at 25 °C.

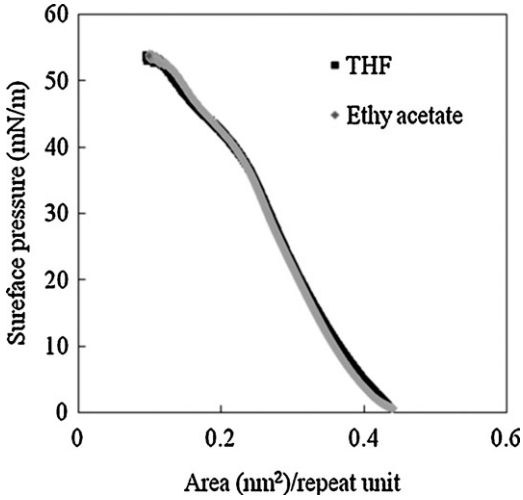

Fig. 7. Surface pressure isotherms at 25 °C obtained with dextran derivative having DS = 150% deposited from THF or ethyl acetate solution (as indicated in the graph).

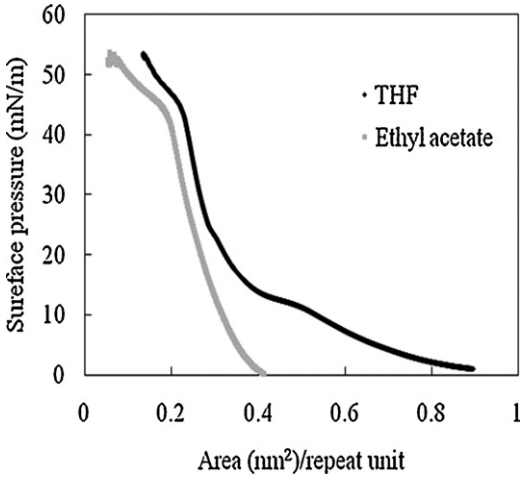

Fig. 8. Surface pressure isotherms at 25 °C obtained with dextran derivative having DS = 113% deposited from THF or ethyl acetate solution (as indicated in the graph).

to get deeper insight into the link between spreading solvent, DS and compression isotherm.

These results show that monolayers deposited from THF or THF:water mixtures are formed by the packing of globular polymer coils with limited contacts with aqueous subphase.

4. Conclusion

Amphiphilic dextrans were prepared using a transesterification reaction catalyzed by lipase AY in dimethylsulfoxide. Polymers recovered after the reaction covered a wide range of degree of substitution between 10 and 150% with the average degree of substitution depending on reaction conditions. Fractionation of modified dextrans was possible by the use of organic solvents differing by polarity and hydrogen bonding ability (ethyl acetate, methanol and water). An overall description of dextran modification during reaction process was proposed based on these results. Finally, adsorption of amphiphilic dextrans at interfaces was demonstrated using interfacial tension measurements (for water-soluble derivatives) and surface pressure isotherms (for non water-soluble polymers).

Self-organization of dextran esters in the presence of an aqueous phase may be used for preparing biodegradable polysaccharide nanoparticles. Indeed highly modified dextrans can be used for forming the core of nanoparticles, while a hydrophilic a superficial layer can be obtained by the adsorption of low modified dextrans. Such nanoparticles would possess adjustable hydrophobicity (varying DS of core polysaccharide) and may be functionalized (thanks to remaining hydroxyl groups both in the core and at the surface). This strategy is currently under investigation and will be reported soon.

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