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New shell crosslinked micelles from dextran with hydrophobic end groups and their interaction with bioactive molecules

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ABSTRACT

Micelles formed in aqueous solution by dextran with hydrophobic (alkyl) end-groups were stabilized through divinyl sulfone crosslinking of the dextran shell. The efficacy of the crosslinking reaction was influenced by the divinyl sulfone amount, the pH and micelle concentration. Crosslinked micelles with a moderate crosslinking degree were further functionalized by attachment of 10 and 17 moles% *N*-(2-hydroxypropyl)-*N*,*N*-dimethyl-*N*-benzylammonium chloride groups along the dextran chain. The size and shape of both crosslinked micelles and their cationic derivatives were analyzed by DLS and TEM. The prepared micelles were able to bind anionic diclofenac (60–370 mg/g), hydrophobic anionic indometacin (70–120 mg/g), and hydrophobic alpha-tocopherol (170–220 mg/g) or ergocalciferol (90–110 mg/g) by hydrophobic or/and electrostatic forces. The release experiments and the antioxidant activity of bound alpha-tocopherol highlighted the potential of the new nano-sized micelles mainly as carriers for prolonged and controlled delivery of hydrophobic drugs.

1. Introduction

Amphiphilic polymers can self-assemble in selective solvents with formation of aggregates of various morphologies, the properties of which recommend them for application in biotechnology, pharmacy, cosmetic or painting industries. Nano-sized aggregates of amphiphilic polymers have been extensively studied as carriers for the controlled drug delivery systems (dos Santos, Medronho, dos Santos, & Antunes, 2013) due to their small particle size which allows the circulation in the blood stream without immobilization at capillaries, and permeate into the target cells through blood vessels. Moreover, these aggregates can solubilize hydrophobic drugs and deliver them to targeted tissues/organs. Amphiphilic block-copolymers represent the main classes of amphiphilic polymers used as hydrophobic drug carriers. Single end hydrophobically modified polymers (amphiphilic semi-telechelic polymers) can associate into spherical micelles, similarly to the amphiphilic block copolymers, and can also be used as drug carriers (Kuskov et al., 2010).

The use of block copolymer or block-like end modified polymers as drug delivery systems can be hampered by the low stability in the blood stream, where the micelles are prone to extensive dilution, which can go below the polymer critical aggregation concentration. Intramicellar crosslinking of either the shell or the core of the core–shell micelles can lead to more stable nanostructured materials (Wooley, 2000). Thus, micelles of triblock poly(propylene oxide)–*b*-poly(glycerol monomethacrylate)–*b*-poly[2-(dimethylamino)ethyl methacrylate] were inner shell crosslinked with divinyl sulfone (DVS) (Pilon, Armes, Findlay, & Rannard, 2006), obtaining shell-crosslinked micelles with amine-functional coronas; micelles of triblock poly(propylene oxide)–*b*-poly[(2-(dimethylamino)ethyl methacrylate]–*b*-poly (glycerol monomethacrylate) were crosslinked in their inner shell with 1,2-bis(2-iodoethoxy)ethane, obtaining shell-crosslinked micelles with hydroxyl-functional coronas (Pilon, Armes, Findlay, & Rannard, 2005). Also, micelles of poly(propylene oxide)–*b*-poly(glycerol monomethacrylate)-(or 2-hydroxyethyl methacrylate)–*b*-2-(diethylamino)ethyl methacylate inner shell crosslinked with DVS can serve as nanoreactors for synthesis of gold nanoparticles (Liu, Weaver, Sauve, & Armes, 2002).

Micelles formed by dextran-block-polystyrene block copolymers were core- or shell-crosslinked with divinyl sulfone (DVS), using different solvent mixtures (Houga et al., 2009). DVS was the crosslinker of choice for preparation of hydrogels from various polysaccharides, such as dextran (Zhang, Bowyer, Eisenthal, & Hubble, 2007), mixture of carboxymethyl cellulose and hydroxyethyl cellulose (Capitani, Del Nobile, Mensitieri, Sannino, & Segre, 2000), hydroxypropyl cellulose (Lu, Hu, & Gao, 2000), hyaluronic

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acid (Ibrahim, Kang, & Ramamurthi, 2010), K-carrageenan (Sagbas, Butun, & Sahiner, 2012) or proteins (Sereikait et al., 2003).

The present study continues our previous work on the synthesis and characterization of dextran modified with hydrophobic (alkyl) end groups, which forms in aqueous solution micelles-like associations above a critical aggregation concentration. The stability of these micelles to dilution could be significantly improved by crosslinking (Nichifor, Mocanu, & Stanciu, 2014). Our aim is the preparation of stable nanoparticles with potential biomedical applications, by the shell crosslinking of the end-modified dextran micelles. As far as we know, the synthesis of crosslinked micelles formed by end-group hydrophobically modified polymers was not reported yet. By varying crosslinking degree one can obtain different shell porosities, which will influence the capacity to encapsulate/release biomolecules. In order to improve the nanoparticle capacity to retain different biologically active molecules, the DVS crosslinked nanoparticles were further chemically modified by attachment of quaternary ammonium groups to the dextran main chain. Applicability of the synthesized nanoparticles as drug carriers was evaluated by encapsulation/release experiments performed with biomolecules of different hydrophobicity.

2. Experimental

2.1. Materials

Dextran with octadecyl end groups (D10-C18) was obtained from dextran with Mr 9000–11,000 (Sigma) by reductive end group amination, according to the procedure described in detail elsewhere (Nichifor et al., 2014). DVS, indometacin (IND), diclofenac (DCF) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were from Sigma Aldrich, alpha-tocopherol (α-TF) and ergocalciferol (ERG) were supplied by Fluka.

2.2. Methods

2.2.1. Synthesis of shell-crosslinked micelles (CM)

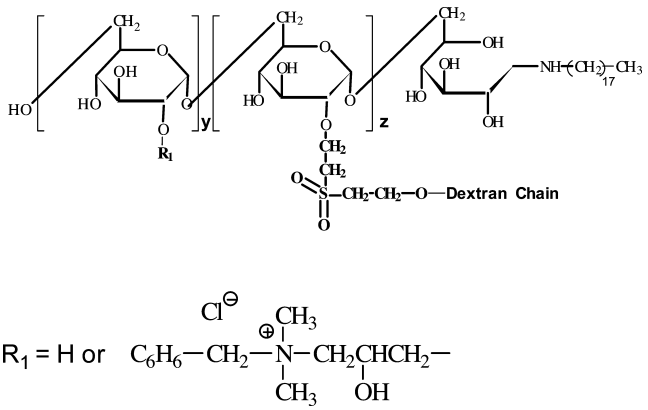
Crosslinking of micelle dextran shell was performed as follows: dry D10-C18 (0.1 g) was dispersed in an aqueous solution of pH 12 (50 mL) at room temperature. The reaction vessel was immersed in an ice bath and the mixture was stirred for 30 min at 700 rpm, then, the calculated amount of DVS was added and the reaction continued for 24 h at room temperature. The reaction mixture was purified by dialysis with a cellulose membrane tube (cut-off 10,000), under conductometric control. The purified reaction product was recovered by lyophilization. Crosslinking extent of the reaction was monitored by sulfur analyses, performed by an EDX—energy dispersive X-ray method. The degree of substitution (DS) with DVS units was calculated with the following equation:

$$DS = \frac{162 \times S\%}{3200 - (118 \times S\%)} \quad (1)$$

where 162: molecular weight of glucopyranosic unit; 118: molecular weight of DVS; *S*%: sulfur content (g%).

Chemical modification of CMs was performed by a procedure described elsewhere (Nichifor, Stanciu, & Simionescu, 2010). Two cationic derivatives were obtained, namely CMQ10 and CMQ17, having 10 and 17 moles% *N*-(2-hydroxypropyl)-*N*,*N*-dimethyl-*N*-benzylammonium chloride groups, respectively.

Chemical structure of the obtained crosslinked micelles is presented in Scheme 1.



Scheme 1. Chemical structure of dextran (or its cationic derivative) hydrophobically modified with octadecyl end groups, crosslinked with divinyl sulfone.

2.3. Characterization of crosslinked micelles

Hydrodynamic diameter and size distribution of crosslinked micelles were determined by dynamic light scattering (DLS) measurements (Malvern Zetasizer NS-Malvern Instruments, UK), using solutions/suspensions with 0.2 g/dL concentration. The reported data are means of 5 separate measurements.

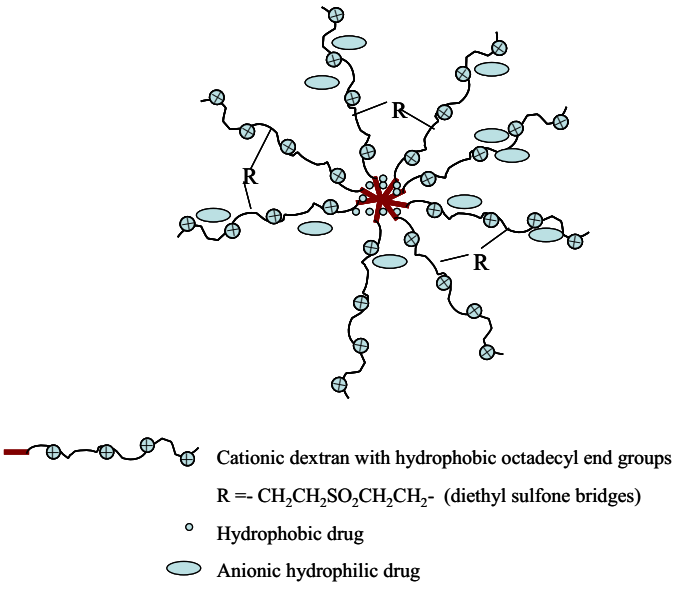
2.3.1. Transmission electron microscopy (TEM)

The size and morphology of dried micelles were evidenced by a HITACHI T7700 transmission electron microscope operated at 120 kV in high resolution mode. Samples with a concentration of 0.2 mg/mL were dropped onto a carbon-coated copper grid, letting the polymer be absorbed for 3 min, followed by filter paper blotting to remove the excess solution. Staining was performed by exposing the samples to 2 wt% aqueous phosphotungstic acid (PTA) for 30 s, followed by filter paper blotting. The samples were dried overnight under ambient conditions and imaged the following day.

2.4. Biomolecules retention/release

Retention of biologically active substances was performed using different retention media, as a function of drug solubility. DCF was dissolved in water, IND in ethanol/water mixture (8/2, v/v), while pure ethanol was used for the preparation of α-TF and ERG solutions. The drug solution with a known concentration (20 mL) was added to 20 mg of either dry particles (in case of DCF and IND) or pre-swollen particles in 2 mL water (for α-TF and ERG). The mixtures were stirred for 48 h at ambient temperature, then they were introduced in dialysis tubes and dialyzed against water (for DCF) or against water/methanol (1/1 v/v) mixture (for IND, α-TF, ERG), until no drug was detected in external fluid by UV measurements performed at 276 nm (DCF), 319 nm (IND), 292 nm (α-TF), and 265 nm (ERG). Finally, the particles were recovered by freeze–drying and the amount of loaded drug was determined by UV measurements of ethanol/water (8/2) suspensions. Drug content was expressed as mg drug/g support. Schematic representation of drug loaded micelles is presented in Scheme 2.

Drug release rate was followed by dialysis. A mixture of drug-loaded particles (30 mg) and PBS 0.1 N, pH 7.4 (3 ml) was placed in a dialyzing tube (cellulose acetate membrane, cut-off: 10,000) and 30 mL PBS was used as an external solution. At pre-determined time intervals, the external solution was removed and replaced with an equal volume of fresh buffer. Each removed solution was analyzed by UV for quantification of the released drug. Cumulative amount of drug release was reported for each drug. For hydrophobicdrugs: IND, α-TF and ERG PBS contained 0.1 g% Tween 80, an emulsifying



Scheme 2. Schematic representation of drug loaded micelles.

agent frequently used for evaluation of hydrophobic drug release (Lee et al., 2012).

2.5. Antioxidant activity

The antioxidant activity of the α-TF retained by micelles was determined by the DPPH method (Ara & Nur, 2009) and compared with that of free α-TF. Briefly, various amounts of samples containing the antioxidant were immersed in a 2 mL PBS buffer and a 1 mL DPPH solution (0.128 g/L methanol). After 30 min, the absorbance of the supernatant at 517 nm was measured. The radical scavenging activity was calculated using the following equation:

$$\text{Scavenging effect } (\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

where A_0 is the absorbance of a standard solution prepared under similar conditions, but containing only DPPH, and A_1 is the absorbance of the supernatant. A lower A_1 value indicates a higher antioxidant effect.

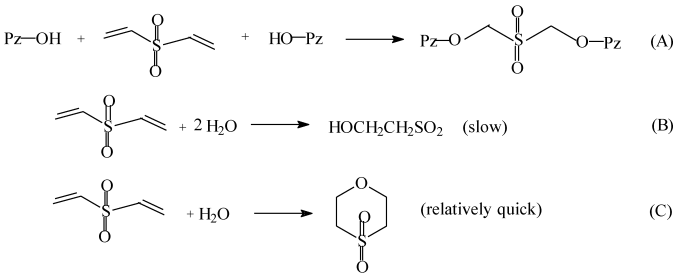
3. Results and discussion

3.1. Synthesis of the crosslinked micelles

3.1.1. Micelle crosslinking

DVS can react with polysaccharides' OH groups resulting in polysaccharide crosslinking. The main reaction (A) follows a Michael addition mechanism, takes usually place in alkaline media under mild conditions, and is accompanied by DVS side reactions [hydrolysis (B) and cyclization (C)] (Scheme 3). The water soluble side products can be removed by dialysis.

According to the previously published studies, the reaction medium alkalinity plays an important role in crosslinking efficacy, which decreases with decreasing pH. The best crosslinking results were obtained at pH 12. At pH 11 only one vinyl group could be linked to polymer, the second one being available for other coupling reactions (Zhang et al., 2007). Our own experiment confirmed these data, as the higher yield and DS with ethyl sulfone bridges were obtained in aqueous solutions of pH 12 (Table 1). The absence of double bonds (at about 6.4–6.6 ppm) in the ^{1}H NMR spectra of the crosslinked micelles prepared at pH 12 (Fig. 1S) supports the double binding of DVS to dextran chain, hence the sulfur content



Scheme 3. DVS crosslinking polysaccharides' OH groups and its side reactions.

is a direct measure of the divinyl sulfone crosslinking extent. At pH ≤ 10 the reaction efficacy was very low. The addition of 1,4-diazabicyclo[2.2.2]octane (DABCO), a non-toxic, inexpensive and highly selective catalyst widely used in organic synthesis (Baghernejad, 2010), improved the crosslinking efficiency at pH 9, without reaching the values obtained at pH 12.

The polymer D10-C18 concentration in reaction medium and the molar ratio crosslinker/glucopyranosic unit (DVS/GU) were also important factors of influence on crosslinking efficacy (Table 2). The crosslinking reaction should be performed at polymer concentrations higher than its critical aggregation concentration [*CAC* = 0.025 g/dL for D10-C18 (Nichifor et al., 2014)], but low enough to reduce inter-micellar cross-linking. At the same ratio DVS/GU, DS increased with increasing polymer concentration from 0.1 to 0.5 g/dL (samples CM6, CM10 and CM14), but at concentrations > 0.2 g/dL the crosslinked micelle size became very high, accompanied by the occurrence of important amounts of big flocks (large aggregates). Medium dilution influenced also the DVS consumption efficiency (expressed as % of bound DVS related to crosslinker amount used in the reaction) due to the increase of the secondary reactions (B) and (C) rates with dilution. For example, the increase of the polymer concentration from 0.1 to 0.2 g/dL resulted in an increase of DVS consumption from 10 to 20%. Higher crosslinking efficiency in more diluted media could be obtained by a significant increase of the DVS/GU ratio (samples CM9 to CM13 in Table 2), the value of which was again limited by occurrence of heavy precipitates (sample CM13) and lost of crosslinked micelle dispersability.

The highest crosslinking rate was observed during the first 2 h, then slowed down (Fig. 1), and reached completion after 24 h.

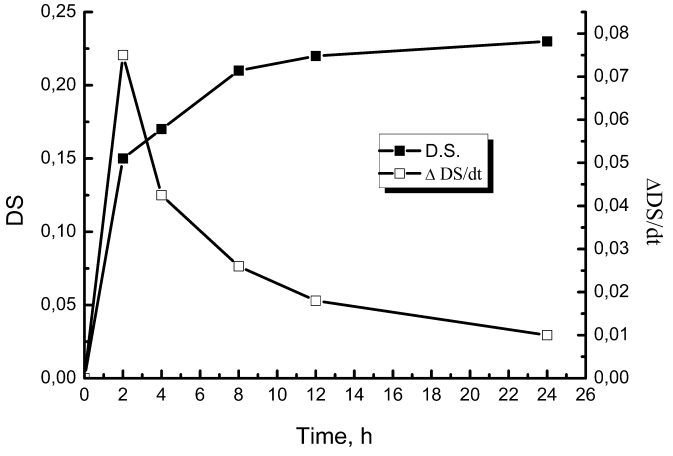


Fig. 1. The influence of reaction time on the degree of substitution (DS) and rate of DVS crosslinking (ΔDS/dt); (molar ratio DVS/GU: 2/1; solution concentration: 0.2 g/dL).

Table 1
Influence of medium pH on the micelle crosslinking reaction. Molar ratio DVS/GU: 2/1; D10-C18 concentration: 0.2 g/dL, 24 h at 700 rpm and room temperature.

Sample	pH	Sulfur content (g%)	Substitution degree (mol%)	Yield (g%) from theoretical	Size (nm (%))
CM1	9	0.05	~0		
CM2	10	0.42	0.02	29	Flocs, 247(75%) 41.6(14%) 20.7(11%)
CM3	11	3.20	0.18	35	540 (45%) 155(35%) 20 (20%)
CM4	12	3.70	0.21	40	257(64%) 18(24%) 58(12%)
CM5	9(DABCO)	2.70	0.15	38	92 (57%) 17 (43%)

Table 2
Influence of D10-C18 concentration and molar ratio DVS/GU on the micelle crosslinking reaction; pH 12, 24 h at 700 rpm and room temperature.

Sample	D10-C18 conc. (g/dL)	Molar ratio DVS/GU*	Sulfur (g%)	DS	Size (nm (%))
CM6	0.1	2/1	2.70	0.15	236 (47%); 19 (29%); 105(23.5%)
CM7	0.1	3/1	3.53	0.20	270 (65%); 35 (35%);
CM8	0.1	4/1	4.35	0.26	280 (74%); 22 (26%)
CM9	0.2	1/1	2.48	0.14	291(80%); 23(16%)
CM10	0.2	2.5/1	3.84	0.23	248(65%); 19.5(29%); 75(5.7%)
CM11	0.2	3/1	5.92	0.38	201(29%); 19(71%)
CM12	0.2	4/1	8.21	0.59	206.6(58%); 17 (42%)
CM13	0.2	6/1	10.34	0.84	Precipitate
CM14	0.5	2/1	7.50	0.52	473 (50%); 22 (45%) flocs

* Glucopyranosic unit.

Taking into account some characteristics required for a good nono-sized carrier designed for controlled drug delivery, a crosslinked micelle sample with moderate crosslinking degree (CM11), which combines the stability to dilution with an advantageous accessibility of the drug inside/out particles, was chosen for further functionalization and drug release studies.

3.1.2. Functionalization of crosslinked micelles

The introduction of ionic groups into the shell of crosslinked micelles can provide new drug carriers with increased application potential due to combination of electrostatic interaction and hydrophobic associations. Pendent *N*-(2-hydroxypropyl)-*N*,*N*-dimethyl-*N*-benzylammonium chloride groups were attached to the dextran main chain using a procedure previously developed for preparation of polysaccharides' cationic derivatives (Nichifor et al., 2010). CM11 sample was used for functionalization and two cationic crosslinked micelle samples (CMQ10 and CMQ17) were prepared and used for biomolecules retention/release studies.

3.2. Crosslinked micelles characterization

Size and size distribution of synthesized particles were determined by DLS measurements and showed that all samples had mainly a bimodal distribution, with the average sizes of the two populations close to those found for D10-C18 micelles, namely about 20 and 200 nm. Some deviations from these values occurred as a function of crosslinking degree and presence of cationic groups. The size of crosslinked micelles decreased with increasing crosslinking degree until a certain value then remained constant (Table 2). In the case of PEO45-GMA40-DEA55 micelles crosslinked with DVS units in the inner shell, their hydrodynamic radius is little changed (Liu et al., 2002). The size and size distribution of crosslinked sample CM11 was almost identical to that of its parent polymer micelles D10-C18 (Nichifor et al., 2014), with two populations representing single micelles and bigger aggregates. This distribution is repetitive, as can be seen from Fig. 2S, which presents the size distribution for several batches of CM11 sample. Also, the size distribution by particles volume and number indicated the preponderant presence of the first population (20–30 nm), both for D10-C18 micelles and for CM11 crosslinked ones; hence crosslinking preserves the size and size distribution of parent micelles. Particle size is an important factor for parenteral route of administration; if the nanoparticles having <10 nm diameter are rapidly cleared by kidney, particles <200 nm are advantageous because can increase blood circulation of an anticancer drug and can efficiently accumulate in tumors through the enhanced permeability and retention effect (EPR effect) (Lee et al., 2012; Beija, Salvayre, Lauth-de Viguerie, & Marty, 2012). Studies on the *in vivo* behavior of these new synthesized crosslinked micelles will be considered in further investigations.

As expected, the size of cationic crosslinked micelles was higher than that of the neutral polymer used for derivatization (CM11) due to electrostatic repulsions between ionic charges along the dextran chains in the shell. For example, the size of the two populations observed in CMQ10 aqueous dispersions was 330 nm (76%) and 42 nm (24%), respectively.

TEM photos of crosslinked micelles and their cationic derivatives reveal the spherical shape of particles and confirm the size range determined by DLS (Fig. 2).

3.3. Interaction of crosslinked micelles with biomolecules

The studied dextran derivatives with cationic and amphiphilic character might be able to retain biomolecules by both electrostatic and hydrophobic interactions. Therefore, biomolecules with carboxylic acidic groups (diclofenac, indometacin) and with pronounced hydrophobic character (alpha tocopherol, ergocalciferol) were chosen for retention/release studies.

Chemical structure of diclofenac, indometacin, tocoferol and ergocalciferol are presented in Scheme 4.

The shell crosslinked micelles containing hydrophobic drugs are mainly designed for parenteral administration; but enteral (oral) administration can be also taken into account for the anti-inflammatory anionic drugs. The most release experiments were carried out under conditions often used for preliminary in vitro studies, in order to evaluate the potential of the new carriers for controlled drug delivery. The most appropriate administration route should be chosen after more extensive *in vitro* and *in vivo* studies.

3.3.1. Antiinflamatory drugs: Diclofenac and indometacin

3.3.1.1. Diclofenac and indometacin retention. DCF [2-(2,6-dichloranilino) phenylacetic acid] and IND [1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-3-indole acetic acid] are nonsteroidal anti-inflammatory drugs used to reduce inflammation and also as an analgesic for reducing pain in certain conditions. The side-effects

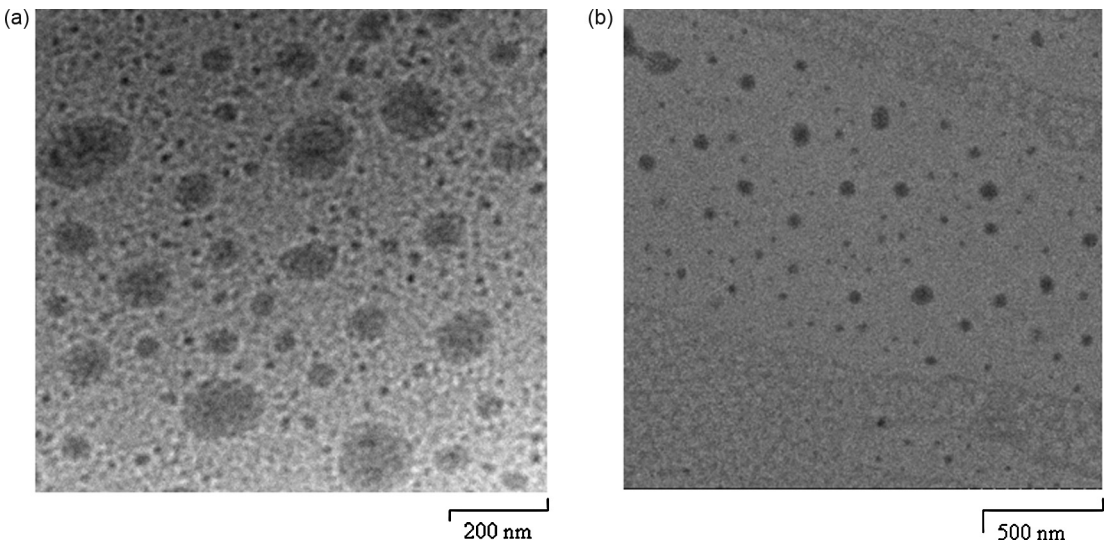
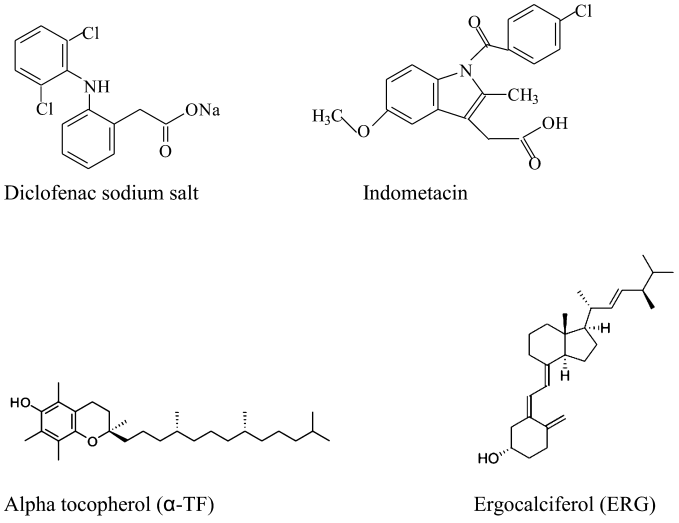


Fig. 2. TEM images of crosslinked (a) and cationic crosslinked micelles (b).



Scheme 4. Chemical structure of the used drugs.

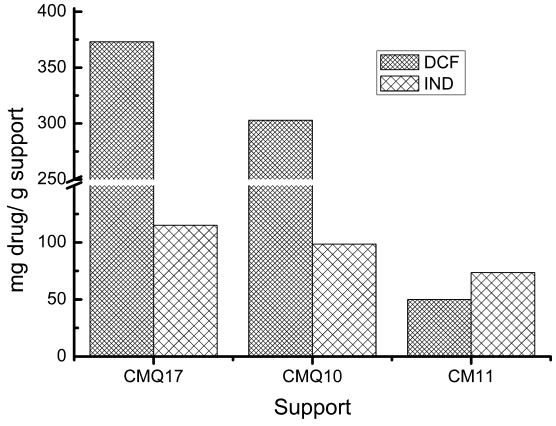


Fig. 3. DCF and IND retention on the studied supports.

observed during the free drugs administration could be overcome by using controlled release formulations. Inclusion of these drugs in the crosslinked micelles might lead to a drug delivery system controlled by both electrostatic and/or hydrophobic forces as well as by a diffusion process.

The results gathered in Fig. 3 show an enhancement of both carboxylic drug retention in the order CM11 < CMQ10 < CMQ17, indicating a preponderance of electrostatic forces in inclusion process. The small but significant retention of these drugs on neutral carrier CM11 suggests the involvement of other interactions, perhaps hydrophobic forces and/or physical entrapment.

There is a difference in affinity of the two drugs for the used polymers. Cationic supports retained IND in much smaller amounts than DCF, but neutral CM11 bound more IND. The differences can be assigned to IND higher molecular weight and hydrophobicity, which may hinder the access to the support ionic groups, but can favor the interaction with CM11 hydrophobic core. The different media used for the retention of DCF (water) and IND (ethanol/water solution 8/2, v/v) can also play a role in the strength of drug interaction with cationic polymers. The cationic dextran chains of the micelle shell will be in a more extended conformation in aqueous medium than in ethanol/water mixture, providing a better access of the charged DCF to the cationic sites. Moreover, the lower dielectric constant of ethanolic mixture can lead to weaker electrostatic interactions than in water.

3.3.1.2. Indometacin and diclofenac release. IND release was studied in PBS solution containing 0.1g% Tween 80 (Fig. 4a). IND release profiles, as well as the release rate dependence on the support chemical structure are similar to those of DCF, even if their water solubility is different, showing that the release process is controlled by the same factors, namely by the electrostatic interactions between cationic groups of the micelles and anionic drug groups. After a "burst" release observed in the first hour, the bound IND was slowly and continuously released over 1 week. The free drug was almost entirely recovered after 48 h. IND release from both cationic and neutral support was slowed down in a more acidic medium (pH 1.2) due to the drug lower solubility (El-Badry, Fetih, & Fathy, 2009) than at pH 7.4 (Nokhodchi, Javadzadeh, Siahi-Shadbad, & Barzegar-Jalali, 2005) and enhanced hydrophobicity as a result of its carboxyl group self-association (Huang, Xiao, & Lang, 2012), leading to a stronger hydrophobic interactions between IND and hydrophobic segments of the micelles' core. Moreover, the particle size decreased in more acidic medium (for example, the average diameter of CMQ10 main population was 330 nm at pH 7.4 and 260 nm at pH 1.2), which can result in a smaller rate of drug diffusion to external fluid.

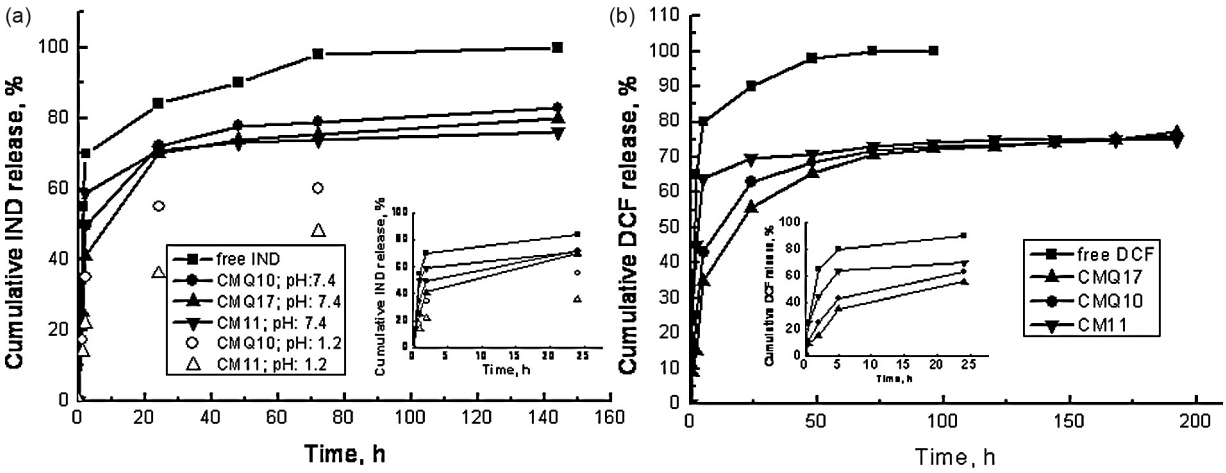


Fig. 4. *In vitro* release of IND (a) and DCF (b) retained by the studied supports. The symbols from insert are the same with those from the main figure.

Diclofenac release in PBS media is presented in Fig. 4b. In these conditions, DCF as free drug exhibited rapid release of about 90% within 24 h, while the DCF-loaded polymeric supports released 60–70% drug in 48 h. The high difference in drug content between cationic and neutral supports (≥300 mg/g and 50 mg/g, respectively) does not allow a direct evaluation of the support chemical structure influence on release rate. However, if we assume that the % of drug released is a measure of the drug partition between the support and the release medium, the data presented in Fig. 4 suggest a higher affinity of the DCF for cationic support, obviously due to electrostatic interactions. Consequently, the cationic support should release the drug with a slower rate than the neutral one, irrespective of the initial drug content. The "burst effect" was more pronounced for the drug retained by hydrophobic forces and/or physical entrapment on the neutral crosslinked support (the small amount of secondary amino groups at end of the polysaccharide chains is not considered). DCF release profile at pH 1.2 is similar to that of IND, due to its lower solubility in acidic pH than at pH ≥ 6 (Bravo, Lamas, & Salomón, 2002) (data not shown).

3.3.2. Hydrophobic drugs alpha-tocopherol and ergocalciferol

3.3.2.1. Alpha-tocopherol and ergocalciferol retention. α-TF, a lypophilic antioxidant is the most biologically-active form of vitamin E. Being fat-soluble, it is incorporated into cell membranes, protecting them against oxidative damage. α-TF has a regulatory effect on either enzymatic activity or gene expression and plays a role in neurological functions.

ERG is a provitamin form of vitamin D, also called vitamin D_2. It is a fat-soluble vitamin that favors calcium and phosphorus adsorption in the body and is used to treat and prevent bone disorders.

Binding of these two drugs can be mainly achieved by hydrophobic interactions with micelles' core, as demonstrated by the data presented in Fig. 5, which show a similar affinity of each drug for both neutral and cationic supports. α-TF was retained in higher amounts than Ergo, possibly due to their different chemical structures. Contrary to ERG with a rather bulky molecule, α-TF has a linear hydrophobic chain, which may favor both the access of the molecules to the inner hydrophobic core and the interaction with similar alkyl chains forming the core.

Drug retention inside nanomicelles can influence their hydrodynamic dimensions, as shown in Table 3 were the size measured by DLS of the unloaded and drug-loaded nanoparticles are presented. Nanoparticles (nonionic and ionic) loaded with α-TF or ERG present higher dimensions than non-loaded ones, as reported also for adriamicin loaded poly(DL-lactide-*co*-glycolide)-grafted pullulan nanoparticles (Jeong et al., 2006), paclitaxel loaded in folated

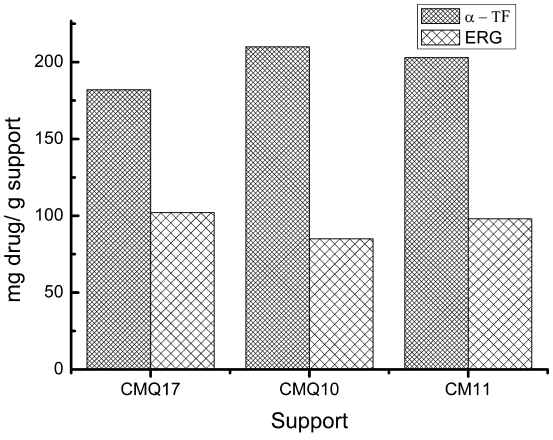


Fig. 5. α-TF and ERG retention on the studied supports.

Table 3
Characterization of drug-loaded crosslinked micelles.

Support	Drug	Drug content (mg/g)	Size (nm (%))
CM11	–	–	201 (29%); 19 (71%)
CMQ10	–	–	330 (76%), 42 (24%)
CM11	α-TF	210	244 (96%); 31 (4%)
CMQ10	α-TF	203	338 (94%); 56 (6%)
CM11	ERG	98	334 (73%); 36 (27%)
CMQ10	ERG	85	380 (91%); 60 (9%)
CM11	IND	74	329 (89%); 36 (11%)
CMQ10	IND	99	292 (97%); 55 (3%)

conjugated chitosan micelles (You et al., 2008), indometacin loaded amphiphilic poly *N*-vinylpyrolidone nanoparticles (Kuskov et al., 2010) and other hydrophobic drugs loaded on self-assembled gelatin nanoparticles (Tran, Tran, & Lee, 2013). Indometacin loaded on nonionic nanoparticles determines an increase of their dimensions, being included by hydrophobic forces into hydrophobic core, while the same drug included in quaternary ammonium group containing nanoparticles determines a decrease of nanoparticles' size, due probably to the electrostatic forces established with the shell ionic groups.

3.3.2.2. Alpha tocopherol and ergocalciferol release in PBS medium, in presence of 0.1% Tween 80 was presented in Fig. 6. The both hydrophobic drugs present a "burst" release in the first 4 h of about 7–15% for α-TF and 14–20% for ERG due probably to the drug adsorbed on the surface of the nanoparticles, followed by a

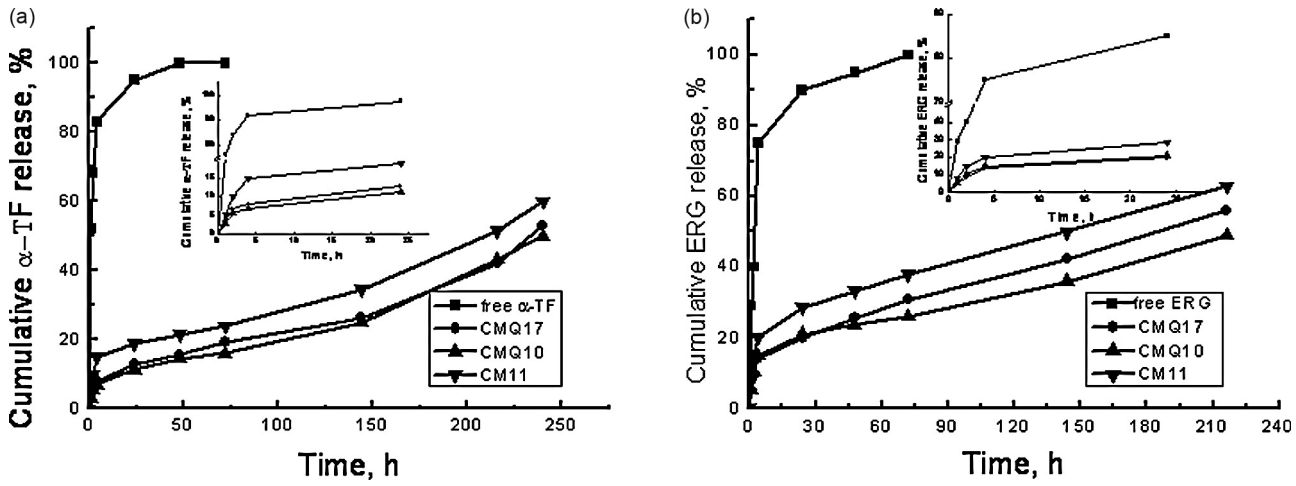


Fig. 6. *In vitro* release of α-TF (a) and ERG (b) retained by the studied supports. The symbols from insert are the same with those from the main figure.

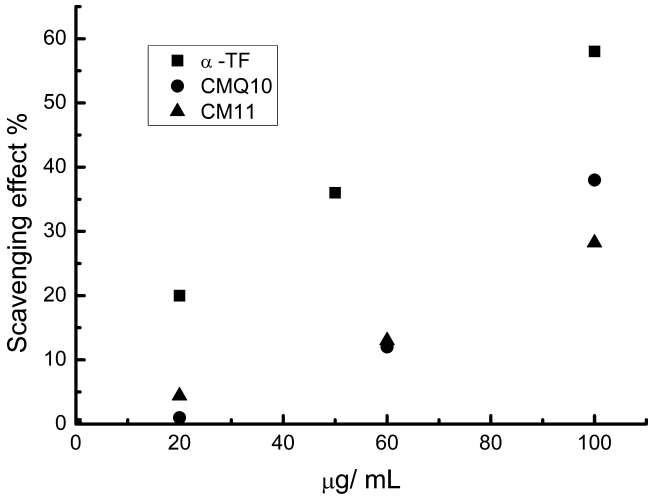


Fig. 7. Scavenging effect of α-TF retained by the studied supports, comparatively with that of free α-TF.

controlled release of 50–60% drugs over 1 week. Comparatively, free α-TF and ERG are released in presence of 0.1% Tween 80 in amounts higher than 80% in 24 h.

Crosslinked micelles both nonionic and cationic present a more pronounced controlled release of hydrophobic drugs (α-TF and ERG), comparatively with more hydrophilic drugs DCF and IND.

3.3.2.3. Antioxidant activity was appreciated for α-TF loaded nanoparticles, comparatively with that of free α-TF. The scavenging effect (which is a measure of the antioxidant activity) of the α-TF loaded particles is lower than that of free α-TF due to the gradual release of the biomolecule retained on the supports (Fig. 7). This behavior can be important in the potential use of the supports for controlled hydrophobic drug delivery.

4. Conclusions

Micelles formed in aqueous solution by dextran with hydrophobic (alkyl) end-groups were stabilized by dextran shell crosslinking with divinyl sulfone. Crosslinked micelles were further chemically modified by attachment of pendant quaternary ammonium groups to dextran main chain. Crosslinked micelles and their cationic derivatives retain biologically active substances by hydrophobic and/or electrostatic forces, as function of their physico-chemical properties, and release them in a controlled manner. The data obtained suggest the potential use of the synthesized micelles mainly as supports for hydrophobic drug delivery systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.11.047.

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