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Synthesis, reactivity, and pH-responsive assembly of new double hydrophilic block copolymers of carboxymethyldextran and poly(ethylene glycol)

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Abstract

Double hydrophilic block copolymers (DHBC) were prepared by end-to-end coupling of two biocompatible water-soluble homopolymers: the polysaccharide dextran (M_w 8300 or 14,700 g mol^{-1}) and ω-amino poly(ethylene glycol) (PEG−NH_2, M_w 3000 or 7000 g mol^{-1}). The synthesis involved, first, specific oxidation of the dextran terminal aldehyde group and, second, covalent linkage of PEG−NH_2 via a lactone aminolysis reaction. The diblock copolymers dextran−PEG (DEX−PEG) were converted in high yield into the corresponding carboxymethyldextran−PEG (CMD−PEG) derivatives with control over the degree of substitution, from 30 to 85 mol% CH_2COOH groups per glucopyranosyl unit. Further modifications of a CMD−PEG block copolymer led to *N*-(2-aminoethyl)carbamidomethyldextran−PEG yielding a pair of oppositely-charged DHBC of identical charge density, chain length, and neutral block/charged block content. The properties of CMD−PEG in aqueous solutions were studied by static and dynamic light scattering as a function of solution pH, providing evidence of the pH-sensitive assembly of the copolymers driven by inter- and intra-chain hydrogen-bond formation.

Keywords: Polysaccharides; Diblock copolymers; Dextran

1. Introduction

Double hydrophilic block copolymers (DHBC) consist of two water-soluble blocks of different chemical nature [1]. They behave like usual neutral or charged hydrophilic polymers in water with no tendency towards association. In some cases, however, a change of temperature, salinity, or pH can turn one hydrophilic block into a hydrophobic one. Micellization ensues via assembly of the newly formed hydrophobic blocks [2–4]. Addition of charged substances to a solution of an oppositely-charged DHBC also triggers micellization, as demonstrated, for example, by Kataoka et al., who employed the resulting micelles as drug and gene delivery systems [5,6]. Moreover, mixing aqueous solutions of oppositely-charged DHBCs with identical neutral blocks results in the formation of polyion complex (PIC) micelles of narrow size distribution which remain stable in water for extended periods of time [7,8]. We reported recently the preparation of DHBCs composed of a natural anionic polymer, the polysaccharide hyaluronan, and a neutral synthetic polymer, poly(ethyloxazoline) [9]. The copolymers were readily soluble in water and underwent micellization in the presence of cationic drugs, such as diminazene. Polysaccharide-based DHBCs are particularly advantageous as components of drug delivery systems in view of their low toxicity and biodegradability.

We report here the preparation of new polysaccharide-based DHBCs consisting of a poly(ethylene glycol) chain linked to carboxymethyldextran (CMD). Dextran is a bacterial polysaccharide composed of α-D-glucopyranosyl units

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predominantly linked by (1 → 6) bonds [10]. It exhibits varying degrees of branching depending on its origin [11]. It is a biocompatible and biodegradable polymer used in various medical applications, such as plasma expander and artificial blood formulations [12,13]. It has been employed also as a component of nanoparticles used as drug delivery vehicles [14–20]. Dextran is highly water soluble and presents no tendency to self-assemble in its native form. Consequently, one needs to modify its structure by incorporation of functionalities which, *per se* or after further treatment, will induce association into nanoparticles or gels [21]. Most synthetic protocols reported to date involve chemical modification of the main chain hydroxyl groups, leading to polyfunctional dextrans with random distribution of functionalities along the chain. It is possible also to modify specifically one chain end by targeting the single aldehyde group located at the reducing end of dextran and subjecting it to reductive amination [22] or oxidation [23]. The aldehyde end group can serve also as an anchoring point to prepare copolymers grafted with dextran chains [24] or to assemble diblock copolymers, having a dextran block [25,26].

Since one of our objectives was to design DHBC for applications in drug delivery, we selected poly(ethylene glycol) (PEG) as the neutral block of the copolymers due to its biocompatibility and its ability to prevent non-specific uptake by the reticuloendothelial system (RES) [27]. Another motivation for the preparation of CMD–PEG DHBC was our interest in understanding the fundamental principles controlling the assembly of block copolymers in which the two blocks are able to undergo non-covalent interactions via hydrogen-bonding. The current understanding of H-bonding driven assembly of DHBC derives primarily from studies of aqueous solutions of poly(methacrylic acid)–poly(ethylene oxide) (PMAA–PEO) copolymers [28–31]. In water of acidic pH, this copolymer forms assemblies via complexation between PMAA and PEO blocks belonging either to two different polymer chains or to the same macromolecule. Aqueous solutions of PMAA–PEO are sensitive to pH changes, displaying up to four distinct association patterns over the 2.5–12 pH range [31]. Diblock copolymers of carboxymethyldextran and poly(ethylene glycol) also possess groups able to undergo H-bonding, thus they are expected to undergo pH-dependent intra- or interpolymeric assembly. Moreover, the carboxymethyldextran block possesses not only carboxylic acid groups as H-bond active groups, but also free hydroxyl groups which may contribute to the formation, stabilization, and pH-responsiveness of CMD–PEG micelles as is the case for interpolymeric complexes between dextran and polycarboxylic acids [32,33].

To achieve these objectives, we prepared a panoply of dextran-based block copolymers using a synthetic route that involves, first, amidation with ω-amino methoxy poly(ethylene glycol) of end-lactonized dextran (DEX–lactone) leading to neutral dextran–poly(ethylene glycol)s (DEX–PEG) and, second, carboxymethylation of the dextran block to produce carboxymethyldextran–poly(ethylene glycol) (CMD–PEG) copolymers of various degrees of modification. The CMD–PEG copolymers can be converted readily to aminated block copolymers, as demonstrated by the synthesis of *N*-(2-aminoethyl)carbamidomethyldextran–poly(ethylene glycol) (ACMD–PEG). All copolymers were characterized in terms of their molecular weight and composition. The aqueous solution properties of CMD–PEG block copolymers were studied as a function of solution pH by potentiometric titrations and light scattering.

2. Experimental

2.1. Materials

Water was deionized with a Millipore MilliQ system. Dichloromethane (CH_2Cl_2) was dried in a solvent purification system provided by Glass Contour. All other solvents were of reagent grade and used as received. ω-Methoxy hydroxy poly(ethylene glycol)s (MeO–PEG–OH) and all other chemicals were purchased from Sigma–Aldrich Chemicals (Milwaukee, WI, USA), unless otherwise stated. The dextran samples were purchased from Fluka Chemical Co. (Buchs, Switzerland) and Amersham Biosciences (Uppsala, Sweden). End group oxidation of the dextrans was carried out following a reported procedure leading to a mixture of δ- and γ-lactone-terminated dextrans [23,34]. ω-Methoxy poly(ethylene glycol)-amine (MeO–PEG–NH_2) [35] and *N*-*t*-BOC-1,2-ethylenediamine [36] were prepared following reported procedures. The molecular properties of the starting polymers are listed in Table 1. Dialysis tubing (SpectraPore, MWCO: 1000 g mol^{-1} unless otherwise indicated) was purchased from Fisher Scientific (Rancho Dominguez, CA, USA). The detailed procedures for the preparation of the diblock copolymers are given as Supplementary data.

2.2. General instrumentation and methods

^{1}H NMR spectra were recorded on a Bruker Avance AMX-400 (400 MHz) spectrometer. Chemical shifts are given relative to external tetramethylsilane (TMS = 0 ppm). FTIR spectra were recorded on a PerkinElmer Spectrum One spectrometer with a resolution of 8 cm^{-1}. Gel permeation chromatography (GPC) measurements were carried out using a GPC system with an Agilent 1100 isocratic pump, a Dawn EOS multiangle laser light scattering detector (Wyatt Technology Corp.) and an Optilab DSP interferometric refractometer (Wyatt Technology Corp.) using PL-aquagel-OH 40 (8 μm) and PL-aquagel-OH 30 (8 μm) columns (Polymer Laboratories) eluted with a pH 7.01 buffer composed of 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, 0.8 mM NaN_3 at a flow rate of 0.5 mL/min. Solutions for analysis had a polymer concentration of 10.0 mg/mL and the injection volume was set at 100 μL. For d*n*/d*c* measurements, stock solutions of each polymer (1.0 mg/mL) in the pH 7.01 buffer were diluted with the same buffer to obtain solutions of concentration ranging from 0.2 to 1.0 mg/mL. UV–vis absorption spectra were recorded on an Agilent 8452A photodiode array spectrometer. Conductivity measurements were carried out with a digital conductimeter with automatic temperature compensation and calibrated

Table 1
Molecular properties of the polymers prepared and yields of the coupling reactions

Polymer[a]	dn/dc[b] (mL/mg)	M_w[c] (g mol^{-1})	M_n[c] (g mol^{-1})	DS[d]	Yield[e] (%)
PEG_{64}–NH_2	0.1295	3000	2800	–	–
PEG_{140}–NH_2	0.1344	7000	6200	–	–
DEX_{140}–lactones	0.1481	8300	6400	–	–
DEX_{68}–lactones	0.1209	14,700	11,000	–	–
DEX_{40}–PEG_{64}	0.1380	9300	7800	–	75[e]
DEX_{40}–PEG_{140}	nd[f]	nd[f]	nd[f]	–	nd[f]
DEX_{68}–PEG_{64}	0.1428	13,200	11,300	–	92[e]
DEX_{68}–PEG_{140}	0.1341	15,900	12,200	–	45[e]
80-CMD_{40}–PEG_{64}	0.1328	12,200	10,200	0.76 ± 0.08	99[g]
85-CMD_{40}–PEG_{140}	0.1328	14,800	10,800	0.86 ± 0.09	98[g]
60-CMD_{68}–PEG_{140}	0.1328	16,700	12,900	0.61 ± 0.06	95[g]
60-CMD_{68}–PEG_{64}	0.1418	16,800	13,400	0.62 ± 0.06	99[g]
30-CMD_{68}–PEG_{64}	0.1378	15,900	12,000	0.31 ± 0.03	95[g]

[a] The prefix denotes the degree of carboxymethylation of the dextran block; the subscript designates the average number of $-CH_2-CH_2-O-$ and glucopyranosyl repeat units of the PEG and dextran samples, respectively.
[b] Values recorded for polymer solutions in aqueous $NaNO_3$ (0.2 M)/NaH_2PO_4 (0.01 M)/NaN_3(0.8 mM); pH 7.01.
[c] From GPC measurements.
[d] Degree of substitution: molar fraction of glucopyranose units carrying a $-CH_2-COONa$ group.
[e] $\text{Yield} = \dfrac{\text{mass recovered (g)}}{\text{mol DEX(lactone)} \times M_n(\text{DEX–PEG})} \times 100\%$.
[f] Crude product converted directly to 85-CMD_{40}–PEG_{140} (see text).
[g] $\text{Yield} = \dfrac{\text{mass recovered (g)}}{\text{mass}(DEX_n\text{–}PEG_m) + (\text{DS} \times (\text{mmol glu}) \times 82\ \text{g/mol})} \times 100\%$.

with Traceable conductivity calibration standard, both supplied by VWR Scientific Products.

2.3. Potentiometric titrations [37]

Solutions for titration were prepared by adding an excess of HCl (0.10 N) to a solution of polymer (CMD–PEG, ~30 mg) in water or acetone/water (1/1 v/v). Titration curves for all polymers were obtained by monitoring the conductivity and pH changes upon addition of standard aqueous NaOH (0.1 M, increments of 50–250 μL). The carboxylate content of the polymers was determined graphically as shown in Fig. S1 (Supplementary data). All titrations were conducted in duplicate. The molecular weight of the dextran samples obtained from GPC analysis was used to determine the carboxylic acid content of the block copolymers.

2.4. Colorimetric assay for the quantitative analysis of primary amines [38]

A solution of PEG–NH_2 or ACMD–PEG (35 mg) in aqueous $NaHCO_3$ (2.0 mL, 2 g/L) was heated to 37 °C and kept at this temperature for 10 min. An aqueous solution of 2,4,6-trinitrobenzenesulfonic acid (TNBS, 147 μL, 50 g/L) was added quickly to the solution and the reaction mixture was kept at 37 °C under stirring for 4 h. The hot solution was treated with aqueous HCl (3 mL, 25 wt.% HCl, 8.2 N). The solution was diluted with water (1/40 v/v). The UV absorbance of this solution at 346 nm was recorded and the NH_2 concentration of the solution was calculated using a calibration curve established by carrying out the same protocol with *n*-butylamine.

2.5. Light scattering studies

Static (SLS) and dynamic (DLS) light scattering experiments were performed on a CGS-3 goniometer (ALV GmbH) equipped with an ALV/LSE-5003 multiple-τ digital correlator (ALV GmbH), a He–Ne laser ($\lambda = 632$ nm), and a C25P circulating water bath (Thermo Haake). The temperature was set at 25 °C. A solution of the diblock copolymer 60-CMD_{68}–PEG_{140} (1 g/L) in 0.1 M NaCl was brought to pH 12. Aliquots of this solution were adjusted to various pH values by addition of 1 N HCl. Solutions were kept at room temperature for at least 2 h after preparation. Prior to the measurements, the solutions were filtered directly into the light scattering cells through 0.45 μm Millex Millipore PVDF membranes.

SLS experiments yield the weight-average molar mass (M_w) of scattering objects in dilute solution, based on the angular dependence of the excess absolute scattering intensity, known as the excess Rayleigh ratio $R(q,c)$ given by Eq. (1):

$$\frac{K(c - c_{mic})}{R(q,c)} \cong \frac{1}{M_w P(\Theta)} + 2A_2(c - c_{mic}) \quad (1)$$

where c is the polymer concentration, c_{mic} is the concentration of micellization onset, q is the scattering vector ($q = (4\pi n/\lambda)\sin(\Theta/2)$), A_2 is the second virial coefficient, n is the refractive index of the solvent, λ is the wavelength of the light in vacuum, and Θ is the scattering angle (30°–150°). The scattering constant is $K = 4\pi^2 n^2 (dn/dc)^2 / N_A \lambda^4$, where d$n$/d$c$ is the refractive index increment and N_A is Avogadro's number. The dn/dc of CMD–PEG in an aqueous 0.1 M NaCl solution was 0.1421 mL/mg. Data were analyzed according to the Berry method, assuming that the macromolecules

are in a swollen conformation. In this case, the particle scattering function is $P(\Theta) = 1 - (q^2R_G^2)/3$, where R_G is the radius of gyration. Since $(q^2R_G^2)/3 \ll 1$, it may be assumed that $1/[1 - (q^2R_G^2)/3] \cong 1 + (q^2R_G^2)/3$. Thus, Eq. (1) becomes:

$$\frac{K(c - c_{mic})}{R(q,c)} \cong \frac{1}{M_w}\left(1 + \frac{R_G^2}{3}q^2\right) + 2A_2(c - c_{mic}) \quad (2)$$

The apparent mass of a polymer ($M_{w,app}$) in a solution of concentration c was obtained by extrapolation of the scattered intensity $R(q,c)/(c - c_{mic})$ to $q = 0$.

In DLS experiments, one measures the normalized time autocorrelation function of the scattered intensity, which can be expressed in terms of the autocorrelation function of the concentration fluctuations. A cumulant analysis was applied to obtain the diffusion coefficient (D) of the scattering objects in solution. Extrapolation of the first reduced cumulant $(\tau q^2)^{-1}$ to $q = 0$ yields the value of D. The hydrodynamic radius (R_h) of the micelles was obtained using Eq. (3),

$$D = \frac{k_B T}{6\pi\eta_s R_h} \quad (3)$$

where η_s is the viscosity of the solvent, k_B is the Boltzmann constant and T is the absolute temperature.

3. Results and discussion

3.1. Synthesis of dextran–poly(ethylene glycol) block copolymers

The neutral dextran–poly(ethylene glycol) block copolymers were obtained by amidation of dextran–lactone with ω-amino methoxy poly(ethylene glycol) (PEG–NH_2) (Fig. 1), a reaction employed previously to prepare various dextran conjugates. The dextran–lactones were obtained by specific end-modification of dextran [34], while the PEG–NH_2 samples were either commercial products or obtained by amination of ω-methoxy hydroxy poly(ethylene glycol) [35]. Analysis by GPC confirmed that the end group oxidation did not affect the molar mass of the polymers (Table 1).

The coupling reaction was carried out in dimethylsulfoxide, a good solvent for dextran–lactone, PEG–NH_2, and the diblock copolymers. In all cases, a large excess of PEG–NH_2 (1/5 molar ratio of lactone to amine) and long reaction times were needed to drive the amidation to completion. The reaction progress was followed by FTIR spectroscopy using the disappearance of the band at 1740 cm^{-1}, characteristic of the lactone group of dextran–lactone [34]. The crude reaction product consisted of a mixture of the desired diblock copolymer and a large amount of PEG–NH_2, which was removed effectively by treatment of the mixture with hot ethanol. The absence of unreacted PEG–NH_2 in the purified diblock copolymers was assessed by a colorimetric test for primary amines [38]. It was confirmed by GPC analysis, as exemplified in Fig. 2, where we present elution profiles of PEG_{64}–NH_2, DEX_{40}–lactone and the resulting diblock copolymer, which eluted with a slightly shorter retention time, compared to DEX_{40}–lactone, indicative of an increase in molar mass. The exact mass of the copolymers was determined by MALLS, using the dn/dc values listed in Table 1. The success of the coupling was confirmed by analysis of the ^{1}H NMR spectrum of the diblock copolymers (Fig. 3) which exhibits two signals characteristic of the methoxy poly(ethylene glycol) chain, namely a strong signal at δ 3.60 ppm and a weak singlet at δ 3.28 ppm due, respectively, to the methylene and methoxy protons, as well as signals characteristic of dextran. The ^{1}H NMR spectrum of each diblock copolymer was compared to that of a mixture of the starting polymers in amounts such that the lactone/NH_2 molar ratio was 1/1. The ratio of the area of the doublet at δ 4.89 ppm, due to the resonance of the anomeric protons, to that of the signal at δ 3.28 ppm, due to the resonance of the methoxy protons, was taken as a gauge of the success of the coupling reaction (Fig. 3). In all cases the ratios recorded for the DEX–lactone/PEG–NH_2 mixtures and for the diblock copolymers were identical within ±2%, the uncertainty of the measurement.

It turned out that the recovery after purification of one diblock copolymer (DEX_{40}–PEG_{140}) was very low (~30%, based on starting DEX_{40}–lactone) as a result of partial solubility of this diblock copolymer in hot ethanol. In this case it was found advantageous to carry out the carboxymethylation on the crude product and to separate excess PEG–NH_2 from the resulting carboxymethylated copolymer (see below).

3.2. Synthesis of carboxymethyldextran–poly(ethylene glycol) diblock copolymers

Conversion of the neutral diblock copolymers into polyanions was achieved by carboxymethylation of the dextran block (Fig. 4). This reaction, routinely employed in polysaccharide chemistry, involves the reaction of monochloroacetic acid (MCA) with dextran under strongly alkaline conditions. The degree of substitution (DS), defined here as the molar% of glucopyranose rings bearing a $-CH_2COOH$ group, varies depending on the reaction conditions [39,40]. To obtain a high substitution level, solutions of DEX–PEG in an 85/15 v/v isopropanol/water mixture were treated with aqueous NaOH (9.0 M) at 60 °C [41]. To achieve moderate carboxymethylation yields, we followed the procedure of Rebizak et al. [42], according to which the reaction was performed in aqueous solution.

The successful incorporation of carboxylate groups onto the dextran block was confirmed by the appearance of a band at 1604 cm^{-1} in the FTIR spectrum of CMD–PEG. It was ascertained further by analysis of the ^{1}H NMR spectrum of the CMD–PEG samples (see Fig. 5 for the ^{1}H NMR spectrum of 80-CMD_{40}–PEG_{64}), which exhibits two signals in the spectral region characteristic of the anomeric proton signals: a signal at δ 4.89 ppm ascribed, by comparison to the spectrum of DEX–PEG, to the resonance of the anomeric proton on glucopyranose rings unsubstituted at C_2, and a signal at δ 5.07 ppm ascribed to the anomeric proton of glucopyranose rings bearing a carboxymethyl group at C_2. It has been shown previously

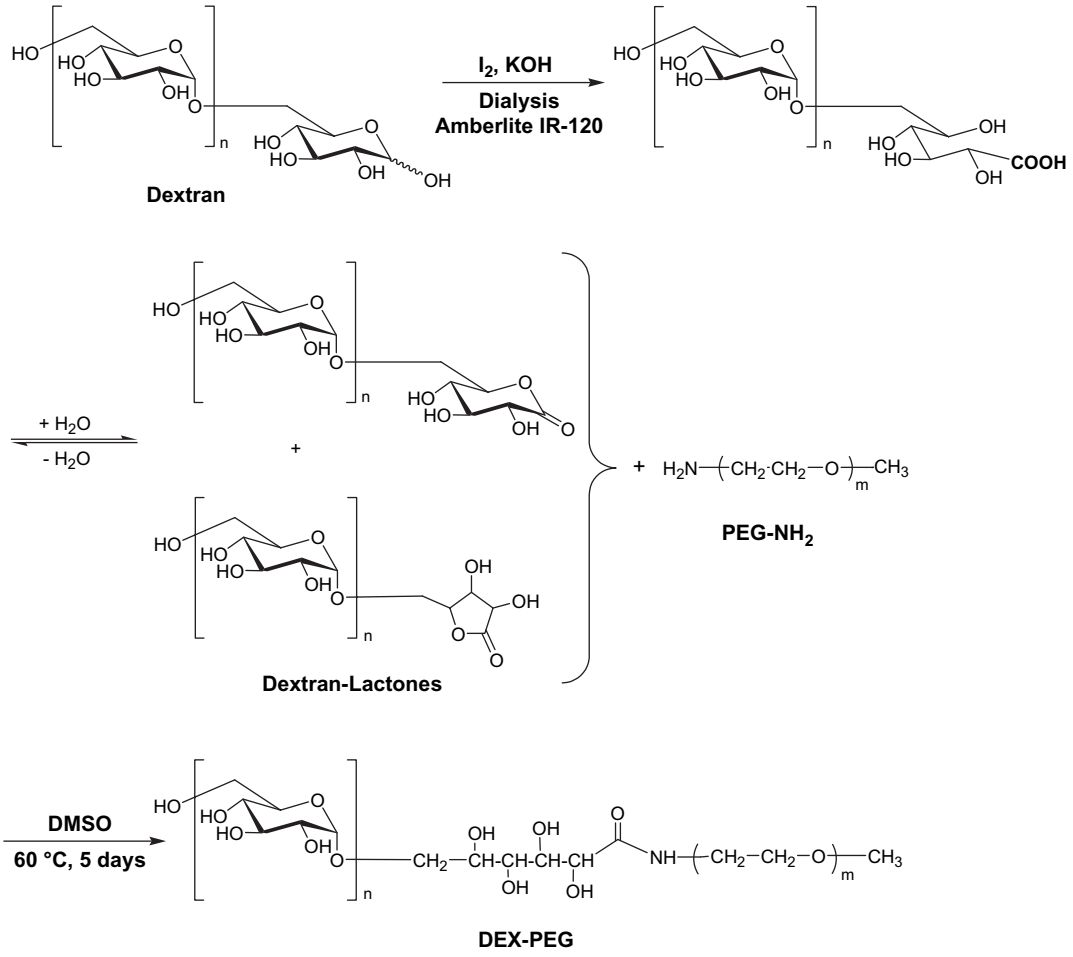

Fig. 1. Synthetic route for the preparation of the dextran–poly(ethylene glycol) (DEX–PEG) copolymers starting from dextran and ω-amino methoxy poly-(ethylene glycol) (PEG–NH_2).

that carboxymethylation of the C_2 hydroxyl group induces a substantial downfield shift on the signal due to the C_1 proton [43]. The ^{1}H NMR spectrum of CMD–PEG samples also presents a series of signals between δ 4.08 and 4.15 ppm, due to the methylene protons α to the carboxylate group. It is expected that carboxymethylation of the dextran block will lead to a mixture of *O*-substituted isomers. In the case of carboxymethylcellulose, modification was shown to occur at all the hydroxyl groups of the glucose ring, although modification of the C_2 hydroxyl was found to be favored kinetically [43]. The complexity of the pattern in the δ 4.08–

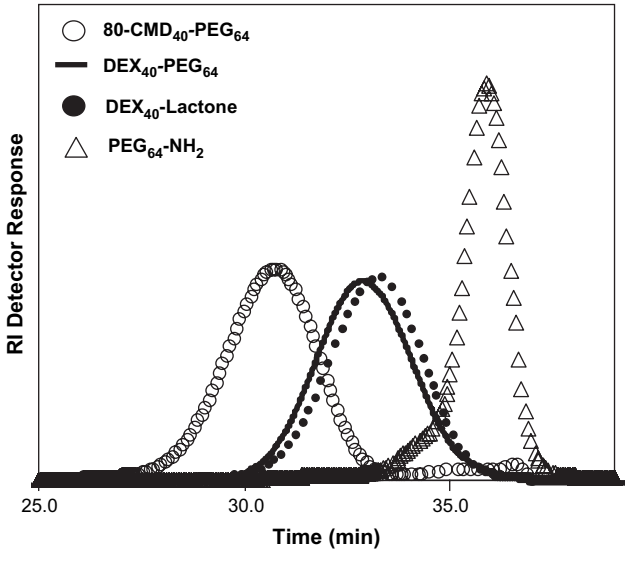

Fig. 2. GPC elution profiles (RI detector, pH 7.02 buffer) of DEX_{40}–lactone, PEG_{64}–NH_2 and the DEX–PEG and CMD–PEG block copolymers obtained from the two polymers.

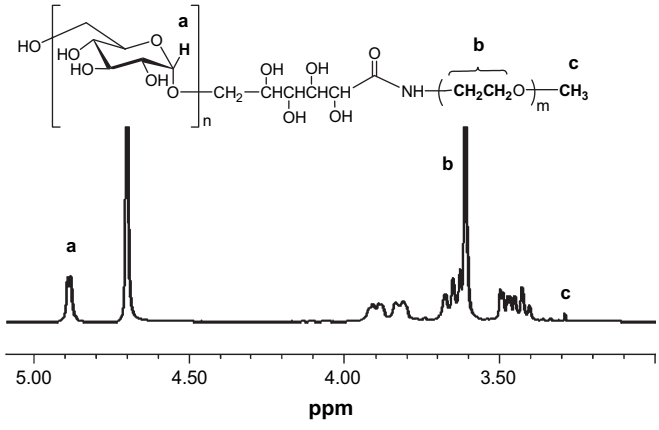

Fig. 3. ^{1}H NMR spectrum of DEX_{40}–PEG_{64} (D_2O, room temperature).

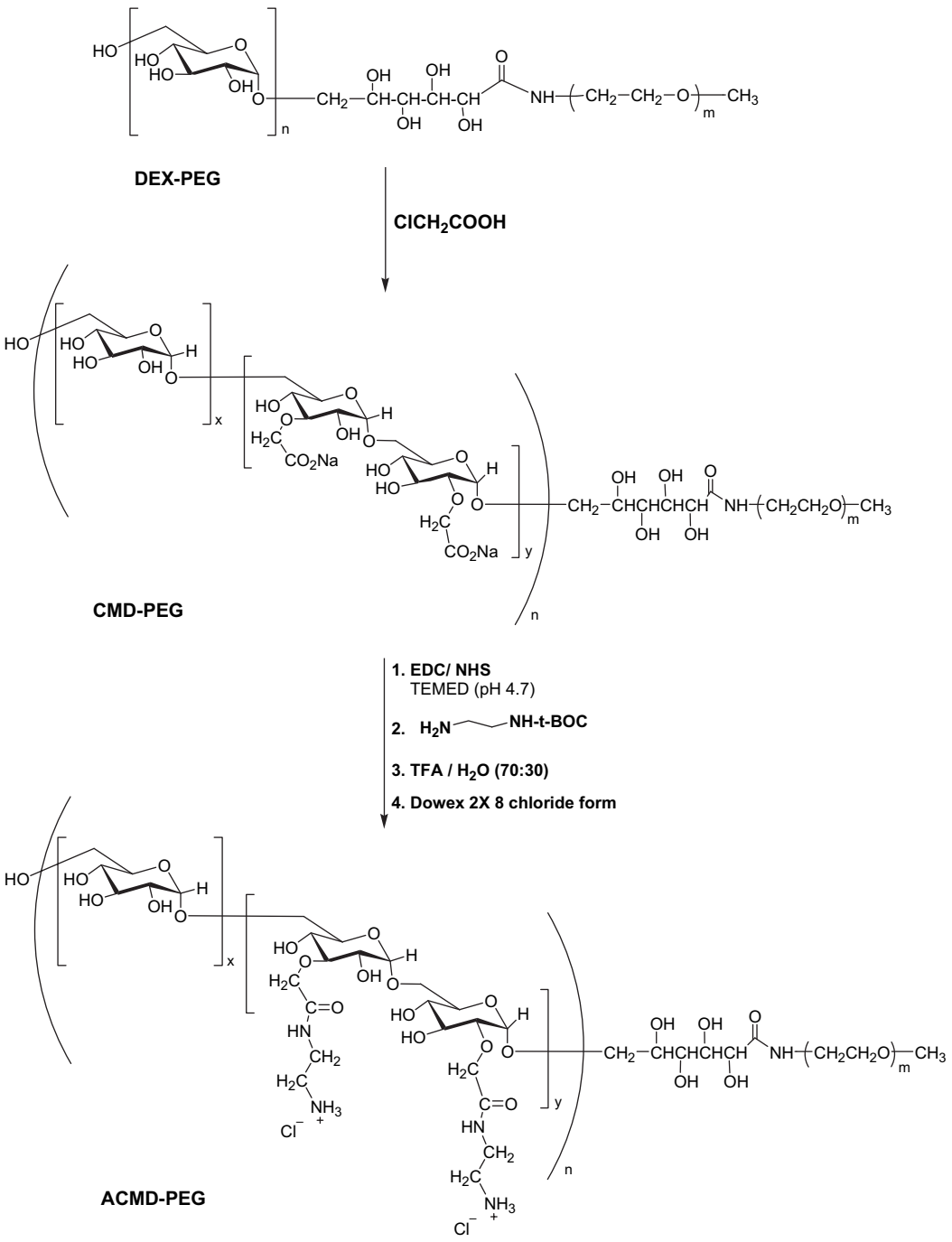

Fig. 4. Synthetic route for the preparation of carboxymethyldextran–poly(ethylene glycol) (CMD–PEG) and *N*-(2-aminoethyl)carbamidomethyldextran–poly-(ethylene glycol) (ACMD–PEG) copolymers; the indices *n*, *x*, and *y* represent, respectively, the total number of glucopyranosyl units, the fraction of substituted glucopyranosyl units in CMD–PEG or ACMD–PEG, and the fraction of unsubstituted glucopyranosyl units in CMD–PEG or ACMD–PEG. The substituted pyranosyl groups are distributed randomly along the CMD or ACMD blocks.

4.15 ppm region of the ^{1}H NMR spectrum of CMD–PEG reflects this heterogeneity in carboxymethylation sites. It has been shown in previous studies that the ratio of the area of the CH_2 signals around 4.0–4.2 ppm to those of the anomeric protons' signals can be used to determine quantitatively the degree of carboxymethylation of cellulose and dextran. In our case, we found that the method was not reliable due to significant spectral broadening. We carried out a potentiometric titration of the samples dissolved in water or a 1/1 v/v water/acetone mixture (Fig. S1, Supplementary data). The degree of carboxymethylation of the CMD–PEG diblock copolymers listed in Table 1 falls into two categories: from ~60% to ~80%, for four samples prepared under conditions favoring high substitution, and ~30% for the sample 30-CMD_{68}–PEG_{64} obtained via the route recommended to attain low substitution levels.

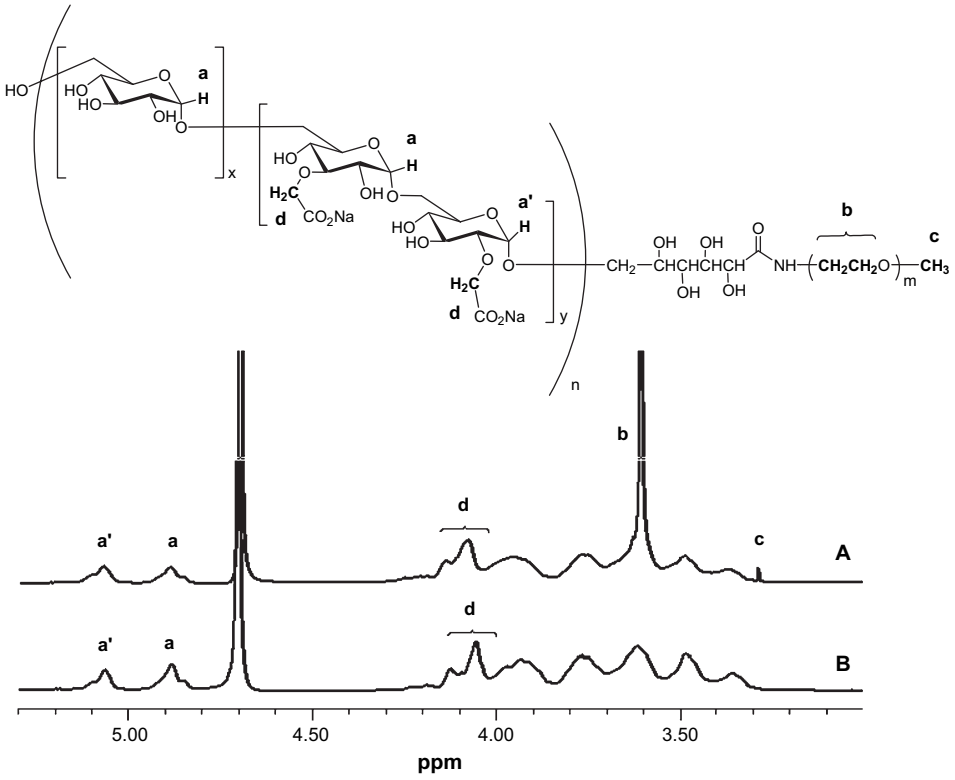

Fig. 5. ^{1}H NMR spectra of 80-CMD_{40}–PEG_{64} (**A**) and 60-CMD_{68} (**B**) (D_2O, room temperature).

It was important to confirm that the amide linkage between the PEG and the dextran withstood the carboxymethylation conditions. One indication that the diblock structure was preserved is provided by the ^{1}H NMR spectrum of CMD–PEG which displays the pair of signals characteristic of the PEG block: a singlet at δ 3.28 ppm due to the methoxy end group of the PEG block and a broad signal at δ 3.60 ppm due to the CH_2 groups. Further proof of the integrity of the diblock structure was gathered from GPC analysis of the CMD–PEG samples, which eluted in shorter times, compared to their DEX–PEG precursors, as shown in Fig. 2 for the sample 80-CMD_{40}–PEG_{64}. The average molar mass of the CMD–PEG diblock copolymers, listed in Table 1, is higher than that of their DEX–PEG precursor as a result of the introduction of carboxymethyl groups. Note that the polydispersity of the mass distribution has not been affected by the transformation.

3.3. Synthesis of N-(2-aminoethyl)carbamidomethyldextran–poly(ethylene glycol) copolymers

Primary amine groups were grafted on the CMD–PEG diblock copolymers by a two-step procedure involving (1) reaction of *N-t*-BOC-1,2-ethylenediamine with the EDC/NHS-activated carboxylic acid groups of CMD–PEG and (2) cleavage of the *N-t*-BOC protecting groups under acidic conditions (Fig. 4). This two-step procedure was favored over the treatment of CMD–PEG with an excess of 1,2-ethylenediamine, reported previously in the case of CMD [42], in order to prevent possible crosslinking reactions. The ^{1}H NMR spectrum of the intermediate *N-t*-BOC protected diblock copolymer presented a singlet at δ 1.35 ppm, characteristic of the resonance of the *t*-butyl methyl protons. From the ratio of the area of this signal to those of the anomeric protons, and knowing the DS of the starting CMD–PEG, we estimated that the conversion yield was >90%.

Deprotection of the polymer with trifluoroacetic acid followed by chloride ion exchange yielded the desired ACMD–PEG diblock copolymer. The FTIR spectrum of this polymer (Fig. 6) presents bands at 1648 cm^{-1} and 1590 cm^{-1} attributed to the amide I and amide II vibration bands, respectively. Analysis of the ^{1}H NMR spectrum of ACMD–PEG confirmed the successful grafting of 1,2-ethylenediamine (Fig. 7). Most significant is the appearance of multiplets at δ 3.33 ppm (signal e in Fig. 7) and 2.33 ppm (signal f in Fig. 7), due to the resonances of the methylene protons α and β to the amide linkage, respectively. In addition, by comparing the signals in the δ 4.08–4.16 ppm range, we note significant differences between the spectra of CMD–PEG and ACMD–PEG, which reflect the changes in the environment of the methylene protons α to the carboxy group (protons d, Figs. 5 and 7) upon amidation of the carboxylate groups. The degree of amidation was determined (1) from the ratio of the areas of the anomeric protons' signals to that of the signal at δ 2.33 ppm and (2) by a colorimetric assay of the amine content. Both methods indicate that the amine content was $27 \pm 1\%$, confirming the high conversion yield calculated from the ^{1}H NMR spectrum of the *N-t*-BOC protected precursor. Thus, the synthetic scheme deployed here

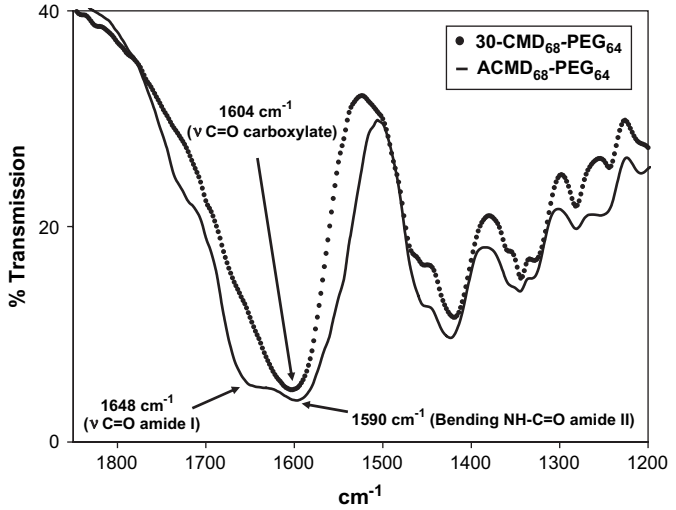

Fig. 6. FTIR spectra of 30-CMD_{68}–PEG_{64} and 30-$ACMD_{68}$–PEG_{64} (powder sample) in the 1800–1200 cm^{-1} wavenumber range.

allowed us to transform CMD–PEG into an oppositely-charged DHBC of identical chain length and charge density.

3.4. Investigation by light scattering of the pH-dependent properties of aqueous CMD–PEG solutions

To gain insight on the association of CMD–PEG in water and to demonstrate the impact of the CMD block pH-sensitivity on micellization, we carried out a set of light scattering experiments with aqueous solutions of 60-CMD_{68}–PEG_{140}. This specific copolymer was chosen, since it has an intermediate level of carboxymethylation (60 mol%) and has a long PEG block, two factors likely to promote H-bond formation not only between PEG and COOH but also between the CMD hydroxyl groups and PEG. Dynamic light scattering, which detects the presence of polymeric micelles and readily provides their hydrodynamic radii (R_h) is the most direct experimental approach to assess the formation of micelles and their disruption in response to a change in pH. Solutions investigated were prepared in aqueous 0.1 M NaCl, to minimize pH-induced changes in the solutions' ionic strength, a factor known to affect the interpolymeric complexation between dextran and poly(carboxylic acids) [44]. DLS confirmed the presence of polymeric micelles in solutions of 60-CMD_{68}–PEG_{140} of pH 2 containing 0.1 M NaCl, with an R_h of 100 nm and a unimodal size distribution. Increasing the pH of the solution induced a decrease in R_h, which reached a value of $\sim$50 nm in solutions of pH >5. The increase in solution pH also changes the size distributions, which are monomodal for solutions of pH <5 ($\mu^2/\Gamma^2 < 0.2$), but above this pH value, the size distributions are bimodal presenting a contribution from small objects ($R_h \sim 3$–5 nm) to the overall correlation function. This contribution increases with increasing solution pH, as depicted in Fig. 8, where we present size distribution profiles recorded for 60-CMD_{68}–PEG_{140} solutions of pH 3, 6 and 12, together with the corresponding autocorrelation functions measured at a scattering angle of 90° (Fig. 8, inset).

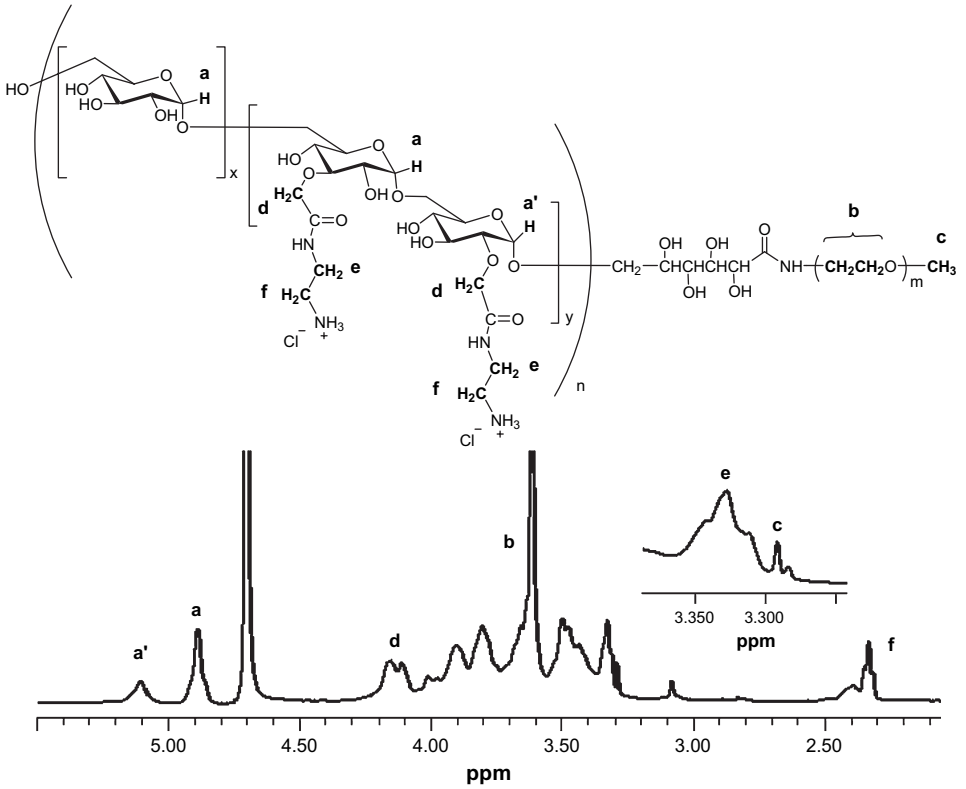

Fig. 7. ^1H NMR spectrum of 30-$ACMD_{68}$–PEG_{64} (D_2O, room temperature).

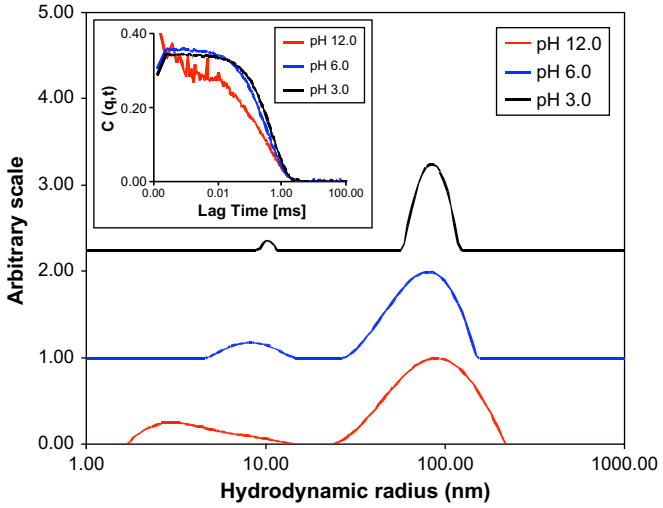

Fig. 8. Size distributions from aqueous solutions of 60-CMD_{68}–PEG_{140} of pH 3, 6 and 12 (0.1 M NaCl, 25 °C); inset: corresponding autocorrelation functions measured at a scattering angle of 90°.

From the apparent molecular weight of the polymers recorded by static light scattering (SLS), it is, in principle, possible to estimate the aggregation number, N_{agg}, of the micelles. From SLS data obtained for acidic and neutral solutions, we estimate that under these conditions, the diblock copolymer 60-CMD_{68}–PEG_{140} forms micelles consisting of 30–40 chains. The intensity of light scattered by basic 60-CMD_{68}–PEG_{140} solutions is very weak, indicating that when the CMD segment of the chain is fully ionized, the assemblies formed contain at most three polymer chains. The formation of such loose aggregates in alkaline solutions of DHBCs containing a PMMA block has been reported previously [45] and attributed to the presence of a small number of un-ionized repeat units. In the case of 60-CMD_{68}–PEG_{140} such assemblies may be attributed to the occurrence of interchain H-bonds between the CMD glucopyranosyl hydroxyl protons and the ether oxygen of the PEG fragments, as depicted in Fig. 9 (right panel). The total scattering intensity for solutions of pH >9 is weak, however, compared to that recorded for solutions of lower pH, indicating that for pH >9, the copolymer exists primarily as isolated chains. In solutions of intermediate pH, the assembly of 60-CMD_{68}–PEG_{140} is driven by the formation of interchain H-bonds between the PEG ether oxygen and both un-ionized carboxylic acid functions and glucopyranosyl hydroxyl groups, leading to loose associates (Fig. 9, center panel). In solution of acidic pH, protonation of the CMD carboxylic acid groups provides additional sites for H-bonding, leading to the formation of tighter objects held together via intra- and inter-chain interactions (Fig. 9, left panel). This interpretation based solely on LS data needs to be confirmed by direct visualization techniques, such as AFM and SEM and by analyses of CMD–PEG samples of different sizes and degrees of carboxymethylation. This study is in progress in our laboratory.

4. Conclusion

A synthetic strategy towards a new family of polysaccharide-based DHBCs has been presented starting from readily available biocompatible homopolymers. The reactions involved are simple, occur in moderate to high yields and do not require elaborate purification or work-up procedures. Neutral, cationic and anionic DHBCs were obtained including pairs of oppositely-charged DHBC of identical neutral block length/charged block length ratio and charge level. A preliminary light scattering study of a CMD–PEG sample in

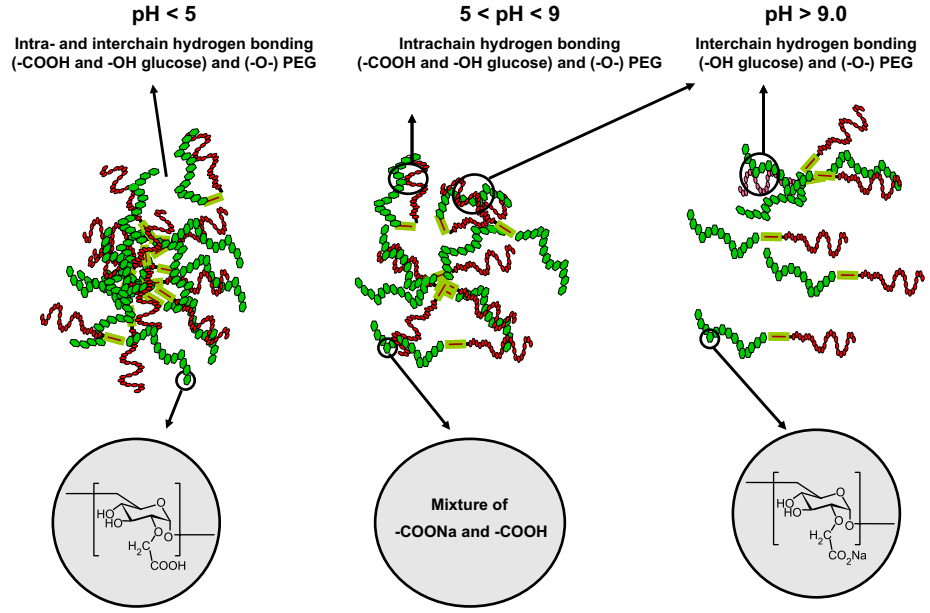

Fig. 9. Pictorial representation of the assemblies formed in aqueous solutions of CMD–PEG block copolymers of various pH values and illustration of the intra- and inter-chain H-bond interactions likely to occur in acidic, neutral and alkaline polymer solutions.

water confirmed that CMD–PEG solutions exhibit a rich pH-responsiveness driven by a combination of H-bond interactions between the polymer functionalities. The diblock copolymers obtained can be ''mixed-and-matched'' to create, via electrostatic and/or hydrogen-bonding interactions, polymeric micelles responsive to changes in pH and/or in ionic strength.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.polymer.2006.12.036.

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