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Non-covalent hydrogels of cyclodextrins and poloxamines for the controlled release of proteins

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ABSTRACT

Different types of gels were prepared by combining poloxamines (Tetronic), i.e. poly(ethylene oxide)/poly(propylene oxide) (PEO/PPO) octablock star copolymers, and cyclodextrins (CD). Two different poloxamines with the same molecular weight (*ca.* 7000) but different molecular architectures were used. For each of their four diblock arms, direct Tetronic 904 presents PEO outer blocks while in reverse Tetronic 90R4 the hydrophilic PEO blocks are the inner ones. These gels were prepared by combining α-CD and poloxamine aqueous solutions. The physicochemical properties of these systems depend on several factors such as the structure of the block copolymers and the Tetronic/α-CD ratio. These gels were characterized using differential scanning calorimetry (DSC), viscometry and X-ray diffraction measurements. The 90R4 gels present a consistency that makes them suitable for sustained drug delivery. The resulting gels were easily eroded: these complexes were dismantled when placed in a large amount of water, so controlled release of entrapped large molecules such as proteins (Bovine Serum Albumin, BSA) is feasible and can be tuned by varying the copolymer/CD ratio.

1. Introduction

Hydrogels are crosslinked polymeric networks with a soft and hydrophilic nature that makes them suitable in numerous biomedical applications such as matrices for drug delivery, wound healing, or tissue engineering (Ko, Shinde, Yeon, & Jeong, 2013). The self assembled hydrogels, i.e. those possessing a physical crosslinking, have attracted much attention lately. These hydrogels can be useful in pharmaceutical and biological applications, especially for some purposes as gene carriers or in the delivery of delicate bioactive agents such as drugs or proteins (Koopmans & Ritter, 2008; Li & Loh, 2008; Li et al., 2006; Lin, Li, & Li, 2013; Ni, Cheng, & Li, 2009; Nielsen, Steffensen, & Larsen, 2009; van de Manakker, Vermonden, van Nostrum, & Hennink, 2008; Van Tomme, Storm, & Hennink, 2008; Wintgens, Daoud Mahammed, Gref, Bouteiller, & Amiel, 2008). Among their promising properties, they present a quick degradation process and the capability of forming the gel "*in situ*" by physical crosslinking between molecules coming from different solutions. In this process of gelation, a protein, a peptide, or a drug can be added to the mixture so it will finally become immobilized inside the bulk of the gel (Gref et al., 2006; Li et al., 2006). This particular property is very significant because it allows us to inject the mixture inside a tissue using a simple needle without any kind of surgery (Li, Ni, & Leong, 2003).

Amphiphilic molecules such as polyethylene oxide/polypropylene oxide (PEO/PPO) block copolymers exhibit interesting self-assembling properties. The behaviour of amphiphilic triblock PEO-PPO-PEO copolymers (poloxamers or Pluronics) can be tailored by modifying the molecular weight and the PEO/PPO ratio within the copolymer (Alexandridis & Hatton, 1995; Trong, Djabourov, & Ponton, 2008). If the distribution of the hydrophilic and hydrophobic blocks is inverted, the resulting PPO-PEO-PPO poloxamers (Pluronic R or reverse) show a different behaviour in water solutions (Zhou & Chu, 1994).

Tetronics or poloxamines are four armed copolymers that contain two blocks per arm, a PEO block and a PPO block. Normal or direct Tetronics present outer PEO blocks, while reverse Tetronics posses inner PEO blocks. The behaviour of normal Tetronics in water has already been reported (Na, Yuan, Stevens, Weekley, & Rajagopalan, 1999) although only a couple of works deal with reverse Tetronics (Larrañeta & Isasi, 2013; Plestil, Pospisil, Sikora, Krakovsky, & Kuklin, 2003). Some Tetronics can form aggregates such as micelles and, in some instances, they can gel under certain conditions, so poloxamines display a great potential as components of drug delivery systems and tissue engineering devices (Alvarez-Lorenzo, Gonzalez-Lopez, Fernandez-Tarrio, Sandez-Macho, & Concheiro, 2007; Gonzalez-Lopez et al., 2008).

In previous works, some authors have discovered that mixing PEO/PPO block copolymers with cyclodextrins (CD) produces self-assembled gels (Koopmans & Ritter, 2008; Li et al., 2006; Nielsen et al., 2009; Tan et al., 2012). Cyclodextrins are natural molecules derived from starch with a relatively hydrophobic cavity, which

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can form inclusion complexes with different molecules, including linear polymers (Dodziuk, 2006). In this case, the resulting complex is called a polypseudorotaxane (Li, Ni, & Leong, 2003; Li et al., 2006; Ni et al., 2009). For instance, α-CD molecules yield inclusion complexes with PEO and its block copolymers (Harada, Okada, Li, & Kamachi, 1995; Harada, Takashima, & Yamaguchi, 2009; Harada, 1997, 1998; Li et al., 2001). On the other hand, cyclodextrins with a wider rim (i.e. β- and γ-CD) are capable of forming complexes with PPO blocks.

Several works regarding the formation of inclusion complexes and gels between poloxamers (Pluronics) and CDs have been published (Li, Ni, Zhou, & Leong, 2003; Li & Loh, 2008; Ni et al., 2009). The complexes between normal poloxamines (Tetronics) and CDs have been recently studied (Simões et al., 2013). In this work, we have used two octablock PEO/PPO copolymers: Tetronic 904 and its corresponding reverse version, Tetronic 90R4. These two medium sized copolymers have practically the same block lengths (16–18 units), but a different architecture. Our main purpose is to ascertain whether the properties of the obtained gels depend on these architectures. After their physical characterization, the gels were tested as controlled release matrices by evaluating their erosion in water media and the release kinetics of a model protein, Bovine Serum Albumin (BSA).

2. Experimental

2.1. Materials

Tetronic 90R4 is a viscous yellow liquid, whose molecular weight given by the manufacturer is M_{90R4} = 6900 g/mol. Its chemical composition was determined by ^{1}H NMR (Bruker DPX 300): $PO_{16}EO_{18}$ per arm (see Figure A1, Supplementary data). Tetronic 904 is a colourless viscous paste with a molecular weight of M_{90R4} = 6700 g/mol. The composition of the blocks was also determined by ^{1}H NMR: $PO_{15}EO_{19}$ per arm. According to their safety data sheets (BASF), these products are non-irritant to the skin, practically nontoxic for dermal applications, and show a low oral toxicity (LD50 > 5000 mg/kg). Both cyclodextrins, α-CD and β-CD, were obtained from Wacker Chemie AG and were used without further purifications. Bovine Serum Albumin (BSA) was purchased from Sigma–Aldrich and used as received.

2.2. Preparation of complexes and gels

All complexes were formed mixing a determinate amount of Tetronic with an aqueous solution of CD followed by vigorous stirring. In the case of poloxamine 904, the copolymer paste was dissolved in a certain amount of water prior to its mixing with the CD solutions, in order to facilitate the homogenization process. Before any measurements, the resulting mixtures were kept at room temperature for at least one night. In the case of the α-CD mixtures, the viscous fluids were used as obtained, without further purifications. On the other hand, for β-CD complexes, a white powder appears at the bottom of the flask when the two solutions are mixed. The supernatant was carefully removed and the powder precipitate was freeze-dried and washed with tetrahydrofuran (THF) in order to remove the excess of uncomplexed poloxamine (Harada, 1998).

2.3. Phase diagrams

A series of Tetronic/α-CD/water gel samples were prepared in 8 mL vials varying the α-CD/copolymer ratio. The samples were placed in a water bath equipped with a thermostatic head. The state of the samples was evaluated for each temperature by direct observation. It was characterized as “gel” or “sol” according to its fluidness when the flask was kept inverted during at least 10 s. Cylindrical 23 mm diameter glass vials containing 5 g samples were used for this analysis. The influence of experimental conditions on the results of this experimental procedure was discussed in a previous work (Larrañeta & Isasi, 2012). The temperature ranged between 10 °C and 60 °C (±0.1 °C), using 5 °C steps; a stabilization time of 10 min was considered as an appropriate delay time prior to testing the mixtures at each temperature.

2.4. Wide-angle X-ray diffraction

The diffractograms of β-CD complexes and α-CD gels were carried out in a Brüker D8 Advance X ray diffractometer equipped with a X ray generator, Kristalloflex K760, using the radiation $K_{\alpha 1}$ of the Cu (λ = 1.5417 Å), and a scanning speed of 0.4° per minute. The α-CD/Tetronic hydrogels were vacuum dried at 60 °C overnight prior to the measurements.

2.5. Viscosity measurements

The viscosity of the α-CD/copolymer hydrogels was evaluated using a Haake Viscotester 550 rotational viscometer equipped with a thermostatic bath Thermo Phoenix II. Two rotors were used: the SV rotor was used for all 90R4/CD mixtures and for those 904/CD mixtures with higher viscosities, and the NV was used for 904/CD mixtures with a low viscosity (gels with 10% and 15% of the poloxamine). All measurements were performed at 25 °C and the range of shear rates covered was between 5 and 40 s^{-1}. After a stabilization time of 30 s, an average of 100 data points was recorded during a measuring period of 60 s.

2.6. DSC analyses

The thermal analysis of the gels was performed using a DSC Mettler TA4000. The samples were kept at 140 °C for 20 min to remove the excess water. Two scans were registered between −100 °C and 140 °C using a scan speed of 20 °C/min. Glass transition temperatures were calculated as the half-height of the corresponding heat-capacity jump. All reported values were determined in the second DSC run.

2.7. In vitro release and erosion kinetics

The release studies were performed from 500 mL of pH 7 phosphate buffer solutions using a SOTAX AT 7 Smart USP dissolution testing device at 37 °C and 25 rpm stirring speed. The gels (20 g) were formed at the bottom of the device vessels by mixing 14.3 g of a 14% α-CD solution (including 150 mg of BSA) and the appropriate amounts of poloxamine and water needed to reach the required gel compositions. Then, the dissolution medium was carefully added to the top at the beginning of the release kinetics. Aliquots (5 mL) were withdrawn according to a sampling time programme of about 10 h, and these sample volumes were replaced with fresh medium. The BSA concentration in each sample was evaluated both by UV–vis (Hewlett Packard 8452A) and fluorescence spectrometries (Perkin Elmer LS 50 B). Both the poloxamine and the substrate (BSA) absorb in the same UV region; in addition, the fluorescence of BSA is quenched by the poloxamine that is also released as the matrices are eroded. Therefore, a combination of the results obtained from these two techniques had to be used in order to determine the amount of substrate released as a function of time (see Supplementary data). α-CD, which is also present in the solution during the erosion process, is not a quencher of BSA. The amount of α-CD released from the matrix was evaluated using size exclusion chromatography (SEC) (Waters 600E system equipped with a Waters 2414 Refractive Index detector and an Aquagel OH-30 column). The

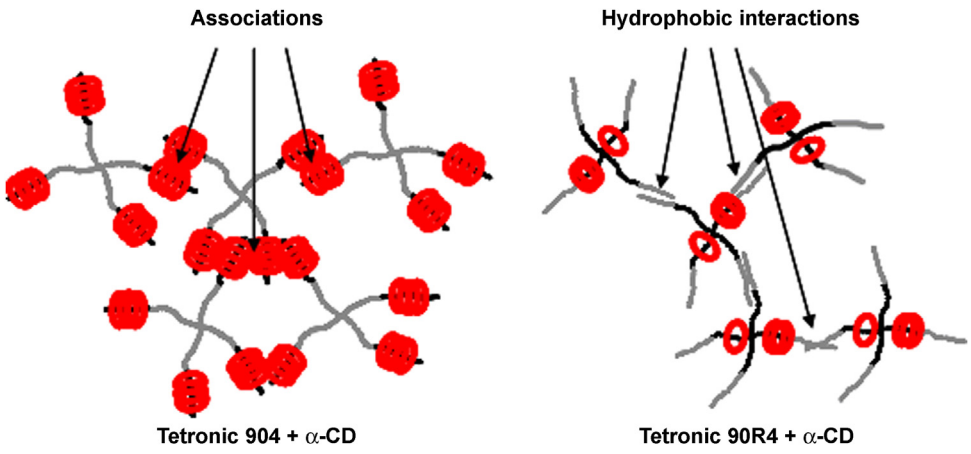

Fig. 1. Associations between α-CD (0) and normal 904 or reverse 90R4 Tetronics.

erosion kinetic curves were constructed evaluating the amount of α-CD dissolved in the samples as a function of time. Acidic and basic media were prepared using HCl 0.1 M or NaOH 0.1 M, respectively.

3. Results and discussion

3.1. Complexation between cyclodextrins and 90R4 or 904

When 90R4 or 904 poloxamine aqueous solutions are mixed with α-CD solutions in certain ratios, a viscous white hydrogel is formed. The evidence of complexation between EO blocks (either in PEO homopolymer or in its copolymers) and α-CD molecules is a well known fact (Larrañeta & Isasi, 2012; Li, Ni, Zhou, et al., 2003; Ni et al., 2009). Both the complexed EO units and the uncomplexed PO blocks tend to self-associate, the first ones by interactions between the CD moieties and the second ones by hydrophobic interactions (see Fig. 1). Hydrophobic associations seem to be more difficult in the case of Tetronic 904 once PEO are threaded due to steric hindrance. On the other hand, associations between PPO blocks threaded by α-CD would be possible in both instances. By varying the α-CD/copolymer ratio and the amount of water, two different types of aggregates can be obtained: either white viscous fluids or gels that remain stable when the flask is inverted. In addition, it has to be pointed out that the formation process is considerably faster for the normal poloxamine (904) than for the reverse one (90R4), a result attributed to the disposition of the PEO blocks in the copolymers. For the reverse poloxamine, the PEO blocks are located in the inner part of the chains so α-CD molecules must surpass the PPO blocks in order to reach the PEO blocks and form the resulting polyrotaxane type structures, as occurs for PPO-PEP-PPO copolymers (Li, Ni, Zhou, et al., 2003). In the case of 904 star octablock copolymers, the PEO blocks are in the outer part of the chains so the kinetics of complexation is faster. For 904 the process takes place in a few seconds, while in the case of 90R4 complexes, they are formed within 20 min.

Figs. 2 and 3 show the sol–gel phase diagrams for the systems 90R4/α-CD and 904/α-CD, respectively (the solubility limit for α-CD aqueous solution is *ca.* 14 wt%). Tetronic 90R4 can not gel by itself in water solutions (Larrañeta & Isasi, 2013; Plestil et al., 2003) in contrast to Tetronic 904, which forms gels under certain conditions of concentration and temperature (Alvarez-Lorenzo et al., 2007) (above 40% wt, see Figure A2, Supplementary data). As can be seen in the phase diagrams, at least 5% (w/w) of α-CD is needed to produce gels for both normal and reverse poloxamines. Interestingly, this threshold value matches the one found for a considerably larger normal poloxamine, Tetronic 908, with $PO_{21}EO_{114}$ per arm (Simões et al., 2013). It is noticeable that, for intermediate α-CD concentrations (5–7%), the gel zone for the reverse Tetronic mixtures corresponds to higher 90R4/α-CD ratios. In contrast, for the direct Tetronic (Fig. 3), the gel zone appears at the left hand side of the diagrams, i.e. for lower 904/α-CD ratios. As can be seen in Fig. 1, the outer PEO blocks can be threaded with higher amounts of cyclodextrin with a low steric hindrance. Then, the interaction between the resulting rotaxane structures is easily achieved through the favourable interactions between CD molecules. For the mixture between 10% of α-CD with Tetronic 904, the phase diagram shows a second gel region at the right hand side of the phase diagram. An additional mechanism for gel formation comes into play: the hydrophobic interaction between the excess of uncomplexed PPO blocks. In the case of the reverse poloxamines, α-CD threads their inner PEO blocks. A few CD moieties (i.e. lower 904/α-CD ratios, left hand side of the diagrams) suffice to disentangle the octablock chains to yield structures that gellify by hydrophobic interactions between PPO blocks. This is indeed the case for reverse Pluronics (PPO-PEO-PPO triblock copolymers) (Larrañeta & Isasi, 2012).

An additional consideration regarding the preparation of the samples is needed. Once the mixtures have been heated to analyze their sol–gel behaviour, they were cooled down to room temperature. In some instances, a second heating scan yielded different results, producing gel-like mixtures instead of fluid sols (see Figure A3, Supplementary data). Nevertheless, these thickening effects can be considered quite subtle for 904 gels and they are not too significant for 90R4 gels either.

When aqueous 90R4 or 904 octablock copolymers are mixed with β-CD solutions, no gels are obtained but solid precipitates. As we have recently shown, the combination of PEO/PPO linear triblock copolymers (i.e. poloxamers or Pluronics) with β-CD leads also to the formation of a crystalline precipitate powder (Larrañeta & Isasi, 2012).

In contrast to the gel formation observed for α-CD and PEO/PPO copolymers, the mixing of the latter with β- or γ-CD aqueous solutions yields white precipitates with a certain crystalline order (Harada et al., 1995; Li et al., 2001). According to the literature, these precipitates are formed by the inclusion of the PPO blocks inside the β-CD cavities producing a polypseudorotaxane type structure (Harada, 1998) yielding self-associated structures such as nanoplatelets (Perry, Hebraud, Gernigon, Brochon, & Lapp, 2011; Tsai, Leng, Jeong, Van Horn, & Wang, 2010).

Although colloidal at first, the β-CD/Tetronic aggregates coalesce in a few minutes. The kinetics of the process depends on the poloxamine and it is faster for the 90R4 (see Supplementary data, Figure A4). In the case of 904/β-CD, the PPO blocks are located in the inner part of the poloxamine arms, so β-CD needs

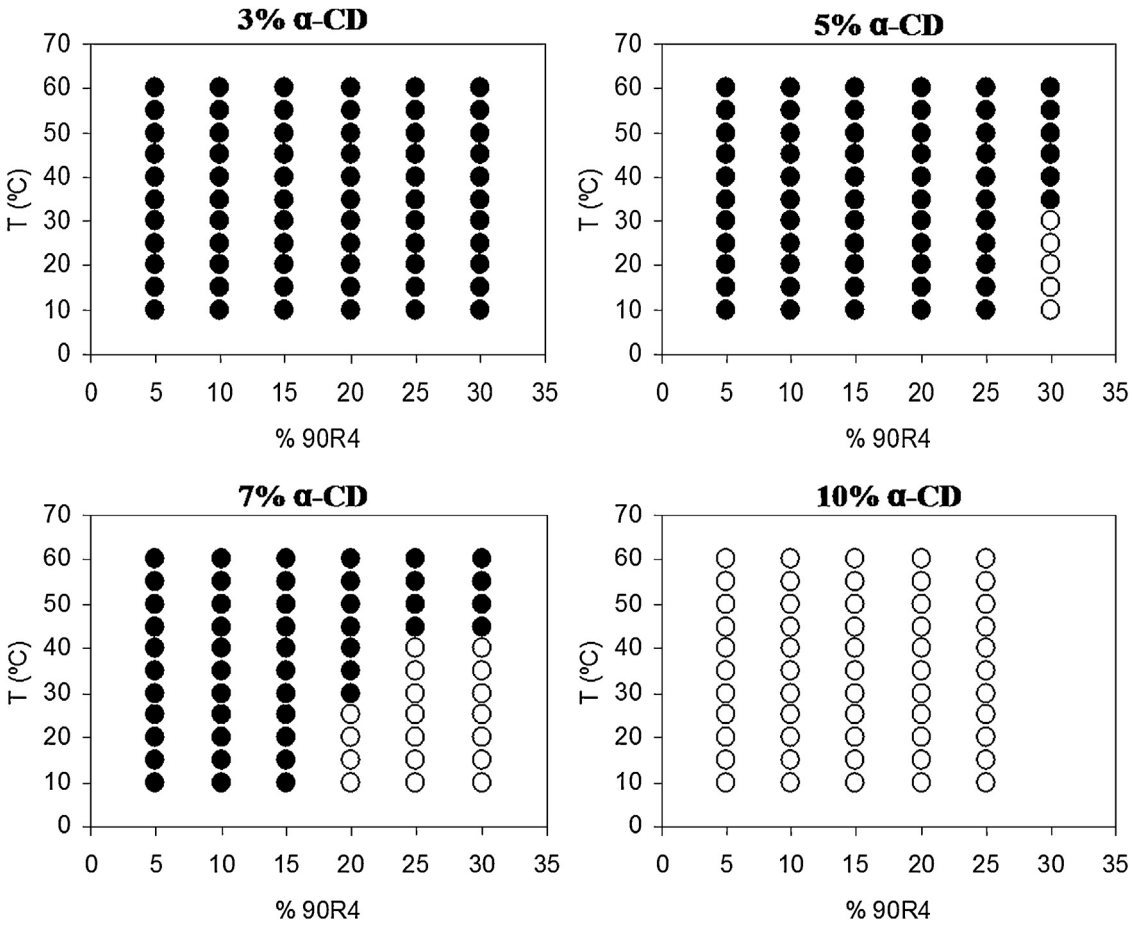

Fig. 2. Temperature–concentration phase diagrams of aqueous reverse Tetronic 90R4 in the presence of α-CD. Sol phase (●) Gel phase (○).

an additional time to pass through the PEO external blocks. These complexes present a crystalline character that can be evaluated by means of X-ray diffraction (see Supplementary data, Figure A5). Using NMR and elemental analysis, it has been found that they posses a defined stoichiometry of about two propylene oxide units per CD molecule (see Supplementary data, Table A1), an optimal value already reported for other PPO polymers and copolymers (Harada et al., 1995). The characterization study of these complexes can be found in the Supplementary data.

When PEO/PPO block copolymers and CDs are combined, different associated systems can be obtained. Using α-CD, two types of associations are present in the mixture: (1) hydrophobic interactions between PPO uncomplexed blocks, and (2) associations between α-CD moieties encapsulating PEO complexed blocks. As a result, firm gels are produced (Larrañeta & Isasi, 2012; Ni et al., 2009). In contrast, the hydrophobic interactions are not present in the mixtures of these PEO/PPO copolymers with an excess of β- or γ-CD because the PPO blocks are complexed and the hydrophilic PEO blocks do not self-associate in aqueous solutions (Li et al., 2006).

Thus, it becomes evident that the type of cyclodextrin determines the gel character of the aggregates. Despite of their unfavourable molecular architecture, the complexes of these star-like copolymers obtained using β-CD are not gelatinous but crystalline precipitates. In contrast, in the case of α-CD/Tetronic systems, the resulting hydrogels can be suitable for sorption and delivery applications. Obviously, these non-covalent interactions can be easily disrupted when the gels or the complexes are in contact with a large amount of water (Li et al., 2001). This behaviour can be proved to be very useful for some applications such as drug release because the gel matrix will be totally eroded in a short period of time. Prior to testing these gels as protein delivery matrices, their physical properties need to be discussed.

3.2. Physical properties of poloxamine/α-cyclodextrin gels

The rheological behaviour of poloxamine/α-CD mixtures has been analyzed using a rotational viscometer. All the samples contain 10% (w/w) of α-CD and different concentrations of reverse 90R4 and direct 904 Tetronic. We have chosen this α-CD composition as a standard value, between the solubility limit of the cyclodextrin (14%) and lower compositions which yield mostly solutions instead of gels (7% and below). These mixtures exhibit a pseudoplastic behaviour, i.e. the viscosity of the samples decreases when the shear rate is increased (see Supplementary data, Figures A6 and A7). Fig. 4 shows the viscosities measured at a constant shear rate of 40 s^{-1} as a function of the copolymer concentration for gels containing the same amount of α-CD (10 wt%). Interestingly, both plots show a cusp at about 20% (w/w) of the copolymer. This copolymer/CD ratio corresponds, in both cases, to four CDs molecules per poloxamine molecule, i.e. a single α-CD molecule per PEO complexing block. This was also the case for a linear PEO/PPO copolymer, Pluronic 10R5, that was previously studied by our group (Larrañeta & Isasi, 2012). Once each PEO block is, on average, threaded to one cyclodextrin moiety, the conformation of the Tetronic arms change, making the self-assembling process feasible. An excess of uncomplexed poloxamine molecules (higher Tetronic/CD ratios) contributes to an increase in the viscosity of the mixture. Thus, it becomes evident why more “ordered” structures, corresponding

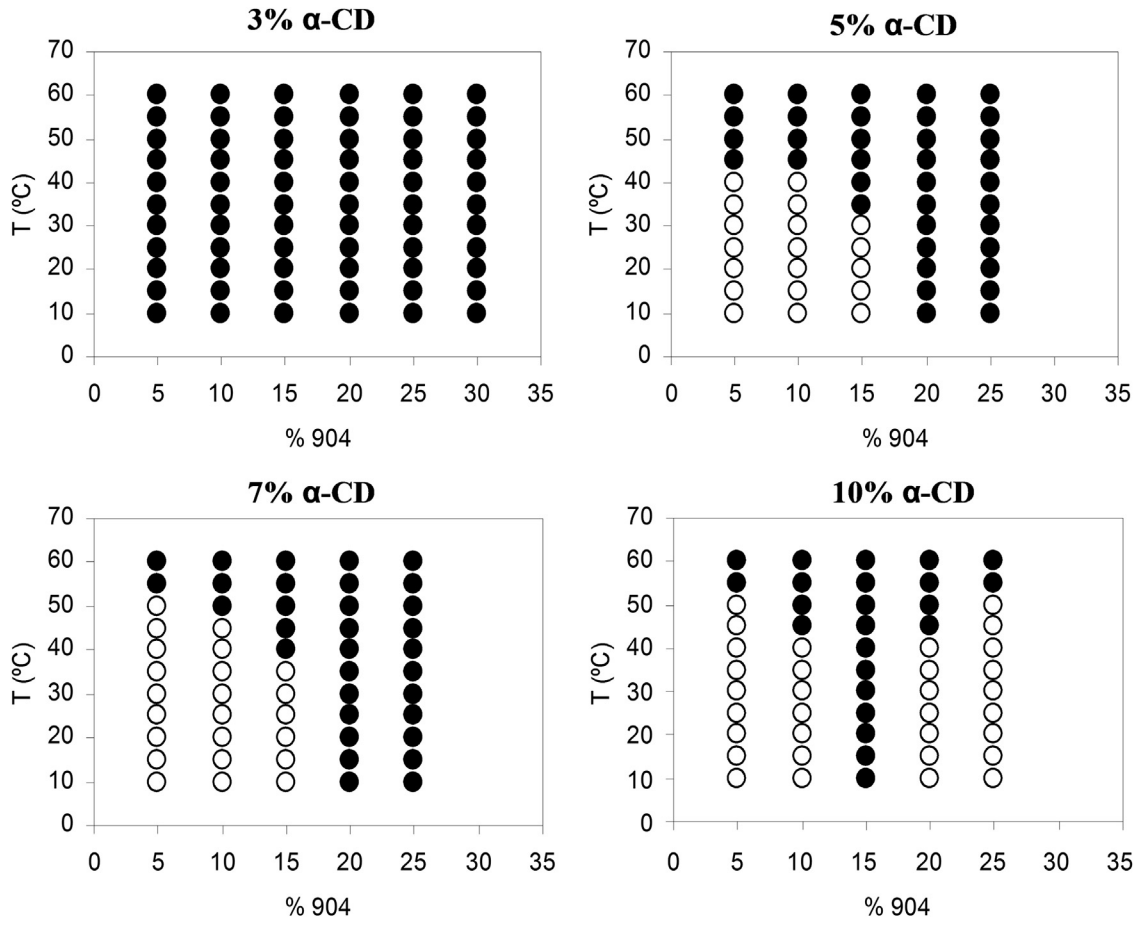

Fig. 3. Temperature–concentration phase diagrams of aqueous normal Tetronic 904 in the presence of α-CD. Sol phase (●) Gel phase (○).

to mixtures with less than a 20% of the poloxamine, yield lower viscosity values.

Although they present the same singular point (cusp), the viscosity range of 90R4 gels is significantly higher than that of 904 gels. The different distribution of the blocks in both copolymers can explain this difference. For 90R4, the outer PPO blocks can establish PPO–PPO intermolecular interactions easily, contributing to a remarkable increase in the viscosity of the bulk. On the other hand, the 904 inner PPO blocks can also establish hydrophobic intermolecular interactions, but in a more restrained way.

A comparison between XRD patterns for different 904/α-CD and 90R4/α-CD gels with the polyrotaxane complex formed between a PEO homopolymer (M_w = 400) and α-CD is shown in Fig. 5. The inclusion complex formed between pure PEO and this cyclodextrin yields a white powder with a channel type structure for α-CD (Harada, Li, & Kamachi, 1992). This structure is clearly detected in the XRD pattern with a characteristic peak located at about 20°, and corresponds to the hexagonal packing (2 1 0) of the channel structure (Chung, Kang, & Kwak, 2007). Fig. 5 shows that the same peak is observed for both 904 and 90R4 gels. Although the amorphous halo is, in both cases, considerably large, the diffraction peaks show that there is some degree of order in the gel structure, attributable to the formation of EO/α-CD complexes that become aggregated

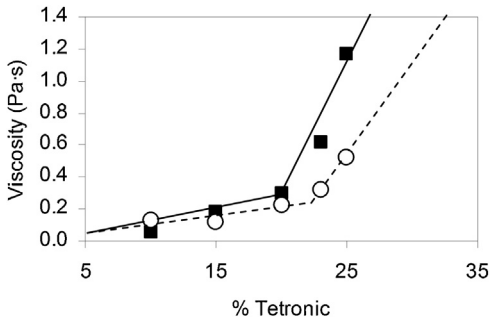

Fig. 4. Viscosities of gels with 10% (w/w) of α-CD and different concentrations of Tetronic 904 (○) and Tetronic 90R4 (■) measured at a constant shear rate of 40 s^{-1}.

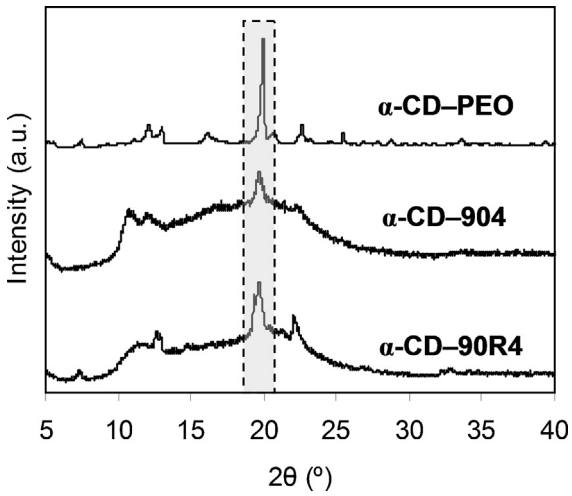

Fig. 5. Wide-angle X-ray diffractograms for α-CD-PEO (M_w = 400) complex, α-CD-904 gel containing 10% (w/w) of CD and 25% of 904, and α-CD-90R4 gel containing 10% (w/w) of CD and 25% of 90R4.

Table 1
T_g for different complexes of 904 or 90R4 with α-CD.

Sample name	Mixture composition (%)			EO/CD	T_g (°C)
	Copolymer	α-CD	H_2O		
α-CD	–	–	–	–	–
T1a10	1.00	10.00	89.00	0.94	–
T2a10	2.00	10.00	88.00	1.90	–
T3a10	3.00	10.00	87.00	2.73	–
T4a10	4.00	10.00	86.00	3.58	−56.5
T10a10	10.00	10.00	80.00	9.04	−55.4
T20a10	20.00	10.00	70.00	18.08	−59.0
904	100	0	0	–	−58.3
TR1a10	1.00	10.00	89.00	0.94	−66.1
TR2a10	2.00	10.00	88.00	1.90	−60.1
TR3a10	3.00	10.00	87.00	2.73	−61.3
TR4a10	4.00	10.00	86.00	3.58	−59.8
TR10a10	10.00	10.00	80.00	9.04	−64.7
TR20a10	20.00	10.00	70.00	18.08	−65.6
90R4	100	0	0	–	−62.8

into ordered domains. It has to be pointed out that both gels were prepared using 25% (w/w) of the copolymer and 10% (w/w) of α-CD, so there is, on average, less than one CD per PEO block in both cases. Thus, the hexagonal packing of the CD units detected by XRD implies side-to-side packing of single rotaxane structures.

The mobility of the chain segments within the block copolymers can be modified because of their complexation with the cyclodextrin moieties (Ni et al., 2009). Table 1 shows the glass transition temperatures for complexes of 904 and 90R4 with α-CD for different poloxamine/CD ratios. As can be seen, the complexes formed between 90R4 and α-CD do not behave in the same way as 904/α-CD ones. It was previously shown that only mixtures formed between linear PEO/PPO copolymers and α-CD corresponding EO/CD mole ratios above 2 show a defined T_g (Ni et al., 2009), (Larrañeta & Isasi, 2012). This characteristic value corresponds to the two EO units that fit inside a CD cavity. When the EO/CD ratio is below 2, all the PEO blocks are threaded within CD cavities. These complexed blocks tend to aggregate into ordered domains so the mobility of the chains is very restricted. Table 1 shows that, for EO/CD ratios higher that 2.7, Tetronic 904 complexes show a defined T_g, similar to that of the pure poloxamine. No glass transitions are detected for mixtures below this ratio (i.e. those with an excess of CD moieties). In contrast, Tetronic 90R4 samples show a clear T_g (with a value close to that of the pure poloxamine) for all the studied EO/CD ratios. Because of their molecular architecture, the complexation of the PEO blocks of 904 is easier than that of 90R4 PEO blocks, located in the inner part of the poloxamine arms. For steric reasons, the reverse poloxamine PEO blocks cannot be fully complexed with CD molecules, so their mobility is not totally restricted and a glass transition is detected.

3.3. Erosion and BSA release kinetics of 90R4/α-CD gels

According to the results shown above, self-assembled hydrogels formed with reverse Tetronic 90R4 and α-CD can be considered as potential matrices for drug delivery systems. These gels showed an appropriate consistency, so erosion kinetic studies were carried out in order to prove if the gels were suitable as release matrices for sustained delivery purposes. On the other hand, the gels based on the direct poloxamine (Tetronic 904) result too soft to be used for these purposes, so they were not tested.

The release of α-CD from the gels can be used as an indicator of the erosion kinetics of the matrix, because α-CD is one of the components of the gel structure. Fig. 6a displays two erosion kinetic curves at physiological pH and temperature for two different 90R4/α-CD gels. One of them (TR15a10), showing a sustained slow erosion kinetics, is composed of 15% of 90R4 and 10% (w/w) of α-CD. The second one (TR25a10) was prepared using a higher amount of the poloxamine, 25%, and the same amount of α-CD, 10%. The latter is more viscous and thicker than the former, as it was shown in Fig. 4, although its erosion is considerably faster. TR15a10 gels contain more CD units per PEO block so the association between complexed PEO blocks is more important than in TR25a10/CD gels. This gel is thicker because of the hydrophobic interactions between its PPO blocks, which yield more viscous aggregates. The disaggregation of polyrotaxane structures in reverse type poloxamers (Pluronics) and poloxamines (Tetronics) is not as fast as the rupture of PPO–PPO interactions because the cyclodextrin molecules must slide across the copolymer chain to break the complexes. Consequently, the erosion kinetics of TR25a10 gels is significantly faster than that of TR15a10.

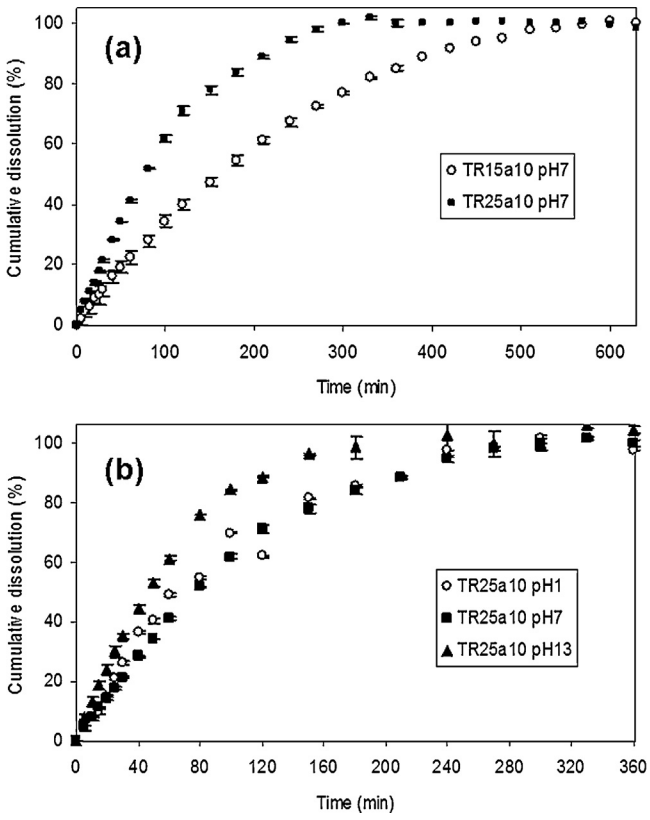

Fig. 6. Erosion kinetics at 37 °C of two mixtures of 90R4 and α-CD, both containing 10% (w/w) of α-CD and 25% (w/w) (■) or 15% (w/w) (○) of 90R4 at pH 7 (a), and three TR25a10 gels at different pHs: 1 (○), 7 (■) and 13 (▲) (b) (error bars for two replicates).

The disaggregation behaviours in different pH media were studied for the same gel (Fig. 6b). The erosion kinetics of three different samples of TR25a10 was evaluated in acidic, neutral and basic solutions. The dissolution of the gels at pH 1 and 7 is nearly the same, despite the fact that Tetronic molecules are protonated at acidic pH, so the interactions between Tetronic molecules should change (Gonzalez-Lopez et al., 2008). Nevertheless, given the size of the poloxamine molecules, it seems to be quite a small effect in this case. In contrast, the sample shows a faster erosion process at pH 13. The main reason for this significantly behaviour may be the ionization of the CD (Maeztu, Tardajos, & Gonzalez Gaitano, 2011). At basic pH, the external hydroxyl groups of the CD molecules are deprotonated. For the PEO complexed blocks, the cyclodextrin rings can start to repel each other, so the stability of the rotaxane clusters decreases.

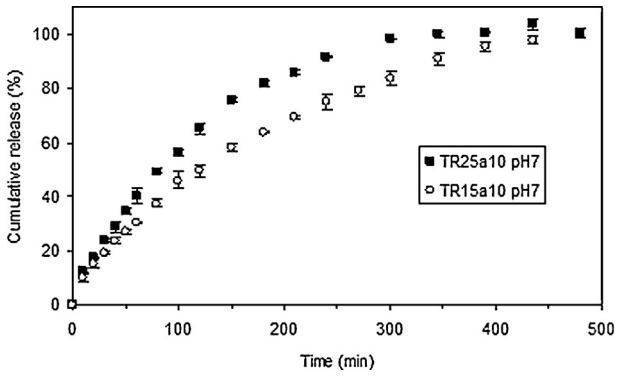

Fig. 7. BSA release from two gels, both containing 10% (w/w) of α-CD and 25% (w/w) (■) or 15% (w/w) (○) of 90R4 at physiological conditions (error bars for two replicates).

The erosion kinetics is almost the same at acidic and neutral pH: by tuning the hydrogel compositions, the dismantling process can occur between 2 and 5 h. A sustained drug release is a promising feature of these systems which should be studied further. To conclude this investigation, we decided to test the release of bovine serum albumin (BSA) as a model protein in simulated physiological conditions. Fig. 7 shows the release of BSA from TR25a10 and TR15a10 gels as a function of time, at pH 7 and 37 °C. The release process of BSA from T25a10 is faster than that of T15a10. This is consistent with the gel erosion profiles. As can be seen, both profiles, i.e. those of the released molecule (BSA) (Fig. 7) and the erosion ones (obtained by measuring the α-CD concentration in the aqueous release medium) (Fig. 6a), are very similar. This indicates that the release of substances from these gels is dominated by the erosion of the gel matrix. As it was expected, a large molecule such as BSA (*ca.* 66.5 kDa) is mainly released as the assembled CD/poloxamines structures are dismantled.

4. Conclusions

Both poloxamines, direct and reverse, can produce supramolecular gels when interacting with α-CD in aqueous media. The comparison between the erosion profiles and the BSA release kinetics for 90R4/α-CD gels proves that they can be applied for sustained release during short periods of time. Although Tetronic 904 gels (i.e. the direct poloxamine) are not firm enough for this study, they could be used for other types of release matrices applications such as ointments. Taking into account that both PEO/PPO copolymers and α-CD seem to be biocompatible, these gels can be promising for biomedical applications.

Besides, it has to be remarked that the viscosity and the gel-to-sol transition temperatures of these mixtures can be tailored by varying the poloxamine/α-CD ratios, so they can be tuned to change at physiological temperatures (see Figs. 2 and 3), in order to be applied for *in situ* gelling. An additional important consequence is that the erosion kinetics of the gel, i.e. its disaggregation speed, can be also tuned by varying the copolymer/α-CD ratio.

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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.2013.11.002.

References

Alexandridis, P., & Hatton, T. (1995). Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: Thermodynamics, structure, dynamics, and modeling. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *96*, 1–46.

Alvarez-Lorenzo, C., Gonzalez-Lopez, J., Fernandez-Tarrio, M., Sandez-Macho, I., & Concheiro, A. (2007). Tetronic micellization, gelation and drug solubilization: Influence of pH and ionic strength. *European Journal of Pharmaceutics and Biopharmaceutics*, *66*, 244–252.

Chung, J., Kang, T., & Kwak, S. (2007). Guest-free self-assembly of alpha-cyclodextrins leading to channel-type nanofibrils as mesoporous framework. *Langmuir*, *23*, 12366–12370.

Dodziuk, H. (2006). *Cyclodextrins and their complexes: Chemistry, analytical methods, applications*. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA.

Gonzalez-Lopez, J., Alvarez-Lorenzo, C., Taboada, P., Sosnik, A., Sandez-Macho, I., & Concheiro, A. (2008). Self-associative behavior and drug-solubilizing ability of poloxamine (Tetronic) block copolymers. *Langmuir*, *24*, 10688–10697.

Gref, R., Amiel, C., Molinard, K., Daoud Mahammed, S., Sebille, B., Amiel, C., et al. (2006). New self-assembled nanogels based on host–guest interactions: Characterization and drug loading. *Journal of Controlled Release*, *111*, 316–324.

Harada, A. (1997). Construction of supramolecular structures from cyclodextrins, polymers. *Carbohydrate Polymers*, *34*, 183–188.

Harada, A. (1998). Polyrotaxanes. *Acta Polymerica*, *49*, 3–17.

Harada, A., Li, J., & Kamachi, M. (1992). The molecular necklace – A rotaxane containing many threaded alpha-cyclodextrin. *Nature*, *356*, 325–327.

Harada, A., Okada, M., Li, J., & Kamachi, M. (1995). Preparation and characterization of inclusion complexes of inclusion complexes of poly(propylene glycol) with cyclodextrins. *Macromolecules*, *28*, 8406–8411.

Harada, A., Takashima, Y., & Yamaguchi, H. (2009). Cyclodextrin-based supramolecular polymers. *Chemical Society Reviews*, *38*, 875–882.

Ko, D., Shinde, U., Yeon, B., & Jeong, B. (2013). Recent progress of in situ formed gels for biomedical applications. *Progress in Polymer Science*, *38*, 672–701.

Koopmans, C., & Ritter, H. (2008). Formation of physical hydrogels via host–guest interactions of beta-cyclodextrin polymers and copolymers bearing adamantyl groups. *Macromolecules*, *41*, 7418–7422.

Larrañeta, E., & Isasi, J. R. (2012). Self-assembled supramolecular gels of reverse poloxamers and cyclodextrins. *Langmuir*, *28*, 12457–12462.

Larrañeta, E., & Isasi, J. R. (2013). Phase behavior of reverse poloxamers and poloxamines in water. *Langmuir*, *29*, 1045–1053.

Li, J., Li, X., Ni, X., Wang, X., Li, H., & Leong, K. (2006). Self-assembled supramolecular hydrogels formed by biodegradable PEO-PHB-PEO triblock copolymers and alpha-cyclodextrin for controlled drug delivery. *Biomaterials*, *27*, 4132–4140.

Li, J., Li, X., Toh, K., Ni, X., Zhou, Z., & Leong, K. (2001). Inclusion complexation and formation of polypseudorotaxanes between poly[(ethylene oxide)-ran-(propylene oxide)] and cyclodextrins. *Macromolecules*, *34*, 8829–8831.

Li, J., & Loh, X. (2008). Cyclodextrin-based supramolecular architectures: Syntheses, structures, and applications for drug and gene delivery. *Advanced Drug Delivery Reviews*, *60*, 1000–1017.

Li, J., Ni, X., & Leong, K. (2003). Injectable drug-delivery systems based on supramolecular hydrogels formed by poly(ethylene oxide) and alpha-cyclodextrin. *Journal of Biomedical Materials Research*, *65A*, 196–202.

Li, J., Ni, X., Zhou, Z., & Leong, K. (2003). Preparation and characterization of polypseudorotaxanes based on block-selected inclusion complexation between poly(propylene oxide)-poly(ethylene oxide)-poly(propylene oxide) triblock copolymers and alpha-cyclodextrin. *Journal of the American Chemical Society*, *125*, 1788–1795.

Lin, Y., Li, L., & Li, G. (2013). A new supramolecular gel via host–guest complexation with cucurbituril and N-(4-diethylaminobenzyl)chitosan. *Carbohydrate Polymers*, *92*, 429–434.

Maeztu, R., Tardajos, G., & Gonzalez Gaitano, G. (2011). Determination of the ionization constants of natural cyclodextrins by high-resolution (1)H NMR and photon correlation spectroscopy. *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry*, *69*, 361–367.

Na, G. C., Yuan, B. O., Stevens, H., Jr., Weekley, B. S., & Rajagopalan, N. (1999). Cloud point of non-ionic surfactants: Modulation with pharmaceutical excipients. *Pharmaceutical Research*, *16*, 562–568.

Ni, X., Cheng, A., & Li, J. (2009). Supramolecular hydrogels based on self-assembly between PEO-PPO-PEO triblock copolymers and alpha-cyclodextrin. *Journal of Biomedical Materials Research Part A*, *88A*, 1031–1036.

Nielsen, A., Steffensen, K., & Larsen, K. (2009). Self-assembling microparticles with controllable disruption properties based on cyclodextrin interactions. *Colloids and Surfaces B: Biointerfaces*, *73*, 267–275.

Perry, C., Hebraud, P., Gernigon, V., Brochon, C., & Lapp, A. (2011). Pluronic and beta-cyclodextrin in water: From swollen micelles to self-assembled crystalline platelets. *Soft Matter*, *7*, 3502–3512.

Plestil, J., Pospisil, H., Sikora, A., Krakovsky, I., & Kuklin, A. (2003). Small-angle neutron scattering and differential scanning calorimetry study of associative

behaviour of branched poly(ethylene oxide)/poly(propylene oxide) copolymer in aqueous solution. *Journal of Applied Crystallography*, *36*, 970–975.

Simões, S. M. N., Veiga, F., Torres-Labandeira, J. J., Ribeiro, A. C. F., Concheiro, A., & Alvarez-Lorenzo, C. (2013). Poloxamine-cyclodextrin-simvastatin supramolecular systems promote osteoblast differentiation of mesenchymal stem cells. *Macromolecular Bioscience*, *13*, 723–734.

Tan, L., Liu, Y., Ha, W., Ding, L., Peng, S., Zhang, S., et al. (2012). Stimuli-induced gel–sol transition of multi-sensitive supramolecular β-cyclodextrin grafted alginate/ferrocene modified pluronic hydrogel. *Soft Matter*, *8*, 5746–5749.

Trong, L., Djabourov, M., & Ponton, A. (2008). Mechanisms of micellization and rheology of PEO-PPO-PEO triblock copolymers with various architectures. *Journal of Colloid and Interface Science*, *328*, 278–287.

Tsai, C., Leng, S., Jeong, K., Van Horn, R., & Wang, C. (2010). Supramolecular structure of beta-cyclodextrin and poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) inclusion complexes. *Macromolecules*, *43*, 9454–9461.

van de Manakker, F., Vermonden, T., van Nostrum, C., & Hennink, W. (2008). Self-assembling hydrogels based on beta-cyclodextrin/cholesterol inclusion complexes. *Macromolecules*, *41*, 1766–1773.

Van Tomme, S., Storm, G., & Hennink, W. (2008). In situ gelling hydrogels for pharmaceutical and biomedical applications. *International Journal of Pharmaceutics*, *355*, 1–18.

Wintgens, V., Daoud Mahammed, S., Gref, R., Bouteiller, L., & Amiel, C. (2008). Aqueous polysaccharide associations mediated by beta-cyclodextrin polymers. *Biomacromolecules*, *9*, 1434–1442.

Zhou, Z., & Chu, B. (1994). Phase behavior and association properties of poly(oxypropylene)-poly(oxyethylene)-poly(oxypropylene) triblock copolymer in aqueous solution. *Macromolecules*, *27*, 2025–2033.