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Stabilization of Pluronic Gels in the Presence of Different Polysaccharides

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ABSTRACT: Several different polysaccharides have been added to pluronic F127 (poloxamer 407) gels to test their ability to stabilize the gels against dissolution in aqueous media over time. The studied polysaccharides include κ-carrageenan, chitosan, hyaluronic acid, pectin, alginate, hydroxyethylcellulose, and ethyl(hydroxyethyl)cellulose. Although all the considered polysaccharides slowed down the dissolution time of the pluronic gels, unmodified polysaccharides only had a modest stabilization effect. However, hydrophobic modification of polysaccharides with a sufficiently long hydrocarbon chain (C_{16}) was found to partly prevent the gels from dissolving for more than 6 months. Shorter hydrocarbon chains did not have the same effect, even at high degrees of hydrophobicity.
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INTRODUCTION

Thermoresponsive polymer gels are interesting for various applications such as controlled drug delivery,[1] flow control in microfluidic devices,[2] sensors,[3] oligonucleotide separation,[4] and tissue-engineering.[5] For drug delivery purposes, systems that are a solution at room temperature and a gel at body temperature are especially interesting. Such a system can easily be injected, and will form a gel *in situ*, which can be used for, for example, sustained release purposes. Since gels that are formed by chemical crosslinking are generally considered to be toxic, it is advantageous to use a system that gels due to physical association forces. Several systems which form a gel at elevated temperatures have been studied such as ethyl(hydroxyethyl)cellulose in the presence of ionic surfactants,[6,7] chitosan in the presence of glycerophosphate,[8] various thermosensitive block copolymers,[9–13] and graft copolymers.[14–16] However, many of these systems have drawbacks such as toxicity, high production costs, or problems with scaling up of the production.

A well-known thermoresponsive gelling system is poly(oxyethylene)-*b*-poly(oxypropylene)-*b*-poly(oxyethylene), PEO-PPO-PEO, triblock copolymers dissolved in water. PEO-PPO-PEO is often referred to as poloxamer or pluronic (which is a trademark of BASF Corporation). These polymers are cheap, easily accessible at large quantities, and approved by the FDA for subcutaneous and intravenous injection. A study on rabbits injected with pluronic samples indicates that doses up to 8.75 mL of 22% pluronic F127 can be administrated to a 70 kg person without inducing hyperlipidemia or altering other blood values.[17] Injected pluronic has been found to be mostly eliminated within 3 days in rats, mainly by renal excretion and also to a smaller extent by biliary excretion.[18]

The central PPO-block of the pluronic chains exhibits an increased hydrophobicity at elevated temperatures. This results in the formation of spherical micelles with a critical micelle concentration (cmc) that is dependent on the composition of the triblock copolymer[19] and on temperature.[19,20] When the polymer concentration is sufficiently high (typically above 15 wt %), the micelles that are formed at elevated temperatures are forming a close-packed cubic lattice leading to the formation of a gel.[21] The gelation temperature of pluronic depends on the length of the PEO and PPO blocks,[22] the pluronic concentration,[22,23] and the addition of salts[23–25] or other additives.[25–28]

Unfortunately, pluronic gels will dissolve within a couple of days when they are in contact with an excess of an aqueous solution.[29–32] This is a severe drawback for long-term drug delivery applications, since the injected gels will dissolve too fast. Attempts of stabilizing the gels by chemically modifying pluronic with other polymers have shown promising results.

Chemical modification of pluronic by hyaluronic acid,[31] chitosan,[33,34] or oligo(lactic acid)[35] made gels that were stable against degradation for at least 20–30 days. Crosslinked gels of pluronic and dextran,[36] or pluronic, poly(ε-caprolactone), and methacrylic acid[37] lasted for at least 2–3 months. Extending the pluronic chain by attaching poly(ether ester urethane)s prolonged the dissolution times of the gels to more than 60 days.[38] Chemically binding the pluronic chains together into longer chains made the gels last for more than 12 days,[29] and for photo crosslinked pluronic gels, for at least 15 days.[39]

A simpler method for stabilization of the pluronic gels is by mixing other polymers into the pluronic samples. However, so far this approach has been much less successful for long-time stabilization than chemical modification. Addition of methyl cellulose or hydroxy propyl methyl cellulose,[32,40–42] carrageenan,[42,43] and laponite,[30] pluronic that was end-capped with oligo(lactic acid)s at both ends,[44] chitosan in combination with sodium tripolyphosphate,[45] and alginate[46] have been studied. Mixing in a different type of pluronic has also been tried.[42] However, even though these additives slowed down the dissolution of the pluronic gels, even the most successful of these additives could only show stabilization against dissolution of the pluronic gels for less than two weeks.

We have examined the ability of several different polysaccharides to stabilize pluronic F127 gels against dissolution, with the aim to prolong the stability of the gels to several months. In addition we have attempted to identify the basic physicochemical properties that are vital for the stabilization process. Of specific interest was the influence of hydrophobic modification of the polysaccharides on the dissolution rates of the pluronic gels.

EXPERIMENTAL

Materials

Pluronic F127 (poloxamer 407), κ-carrageenan, and octaethylene glycol monohexadecyl ether were obtained from Sigma-Aldrich. Hyaluronic acid (sodium hyaluronate, Pharma grade 150), was obtained from NovaMatrix, FMC Biopolymer, Norway. Chitosan (chitosan chloride, PROTASAN™ UP CL 213) was purchased from NovaMatrix, FMC Biopolymer, Norway. Low methoxylated pectin, LM-pectin (Genu® pectin LM12 CG-Z) and high methoxylated pectin, HM-pectin (Genu® pectin 150 USASAG) were obtained from CPKelco, Großenbrode, Germany.

Hydroxyethylcellulose (HEC; Natrosol 250 GR) was kindly donated by Hercules, Aqualon Division. Analogous hydrophobic samples, containing alkyl chains grafted to the polymer backbone, were prepared using a previously described procedure:[47,48] A glass separable reactor equipped with a mechanical stirrer, thermometer, and a condenser tube was charged with HEC and isopropyl alcohol, and an aqueous solution of sodium hydroxide was added to prepare a slurry. The slurry was stirred at ambient temperature for 30 min in a nitrogen atmosphere. Glycidyl hexadecyl ether was added to conduct a reaction at 80°C for 8 h and thereby accomplish hydrophobization of the polymer. After completion of the hydrophobization reaction, adding acetic acid neutralized the liquid reaction mixture.

Alginate (LF 10/60 LS), was supplied by FMC Biopolymers, Norway. Analogous hydrophobic samples, with alkyl chains grafted to the polymer backbone, were prepared using a previously described procedure.[49] An aqueous solution of sodium alginate was adjusted to pH = 3.4 by addition of 0.1*M* HCl, and the polymer concentration was diluted to 2.0 wt %. An aqueous solution of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl) was added to this solution. After 5 min of reaction, octylamine was added and the mixture was stirred for 24 h at ambient temperature.

All the ethyl(hydroxyethyl)cellulose (EHEC) samples were obtained from Akzo Nobel Surface Chemistry AB, Stenungsund, Sweden. The hydrophobically modified (HM) EHEC samples are equivalent to $EHEC_{0.8}$, except that hydrophobic side chains are grafted to the anhydroglucose units. For the HM4-0.7C_{12}-$EHEC_{0.8}$ sample, there is a spacer of 4 ethylene oxide (EO) groups between the hydrophobic alkyl chains and the polymer backbone, while no spacer group is used for HM0-0.7C_{12}-$EHEC_{0.8}$.

Pluronic F127, κ-carrageenan, octaethylene glycol monohexadecyl ether, hyaluronic acid, and chitosan were used without further purification. The remaining polymers were dialyzed against Millipore water and thereafter freeze dried before use.

A summary of the characteristic data for the polymers is provided in Figure 1, together with a schematic representation of the repeating units of the polysaccharides, and the structure of pluronic and the C_{16} surfactant (octaethylene glycol monohexadecyl ether).

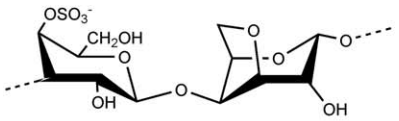
A phosphate buffer saline (PBS) was used for the preparation of the samples. The buffer was prepared by mixing 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, 8 g NaCl, and 0.2 g sodium azide. Milli-Q water was added to a total volume of 1.0 L, and the pH was adjusted with acetic acid to 7.4. The chemicals used for making the buffer solution were purchased from Sigma-Aldrich.

Sample Preparation and Dissolution Studies

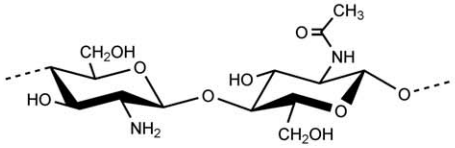
17.5 wt % Pluronic was added to PBS, and stirred at 10°C until completely dissolved. 0.5 wt % of each of the various polysaccharides was added directly into the pluronic solutions, and stirred at 10°C until completely dissolved, giving a final concentration of 17.4 wt % pluronic and 0.5 wt % polysaccharide. All the samples were solutions at 10°C and formed gels at 37°C; 5 g of each sample was weighed into 20 mL glass vials, leaving an exposed top surface of 4.9 cm^2. The samples were placed in an incubator at 37°C. 15 mL of PBS (37°C) was placed on top of each gel, and the gels were kept at 37°C over a period of time. At various intervals, the solution on top of the gels was completely removed, and the remaining gels weighted. Afterwards, 15 mL of fresh PBS (37°C) was placed on top of each gel.

RESULTS AND DISCUSSION

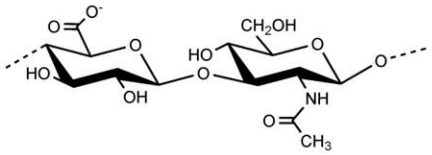
Figure 2 illustrates the time it takes for the pluronic gels to dissolve in PBS at 37°C. The pure pluronic gels dissolved completely within 40 h. This is in agreement with previous findings.[30–32] As observed previously for similar systems,[32,40,41,43] it is evident that for all of the studied



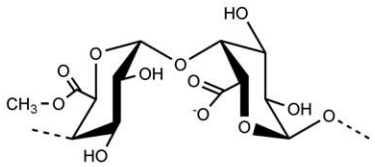
κ-carrageenan



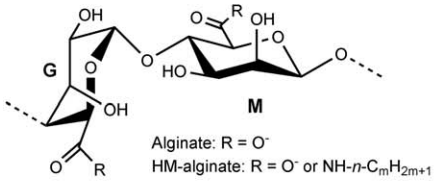
Chitosan
DDA = 83 %.
$M_w = 3.1\times10^5$; $M_w/M_n = 2.7$.[51]



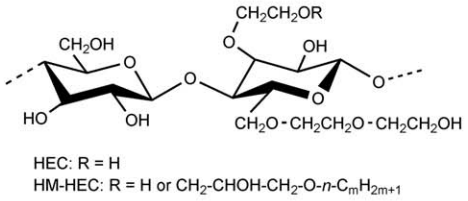
Hyaluronic acid
$M_w = 1.9\times10^6$; $M_w/M_n = 1.1$.[52]



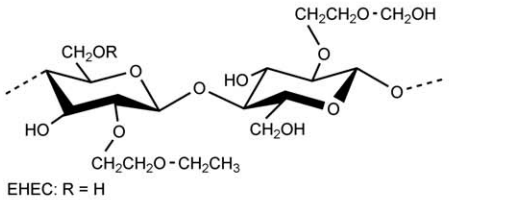
LM-pectin
DE=34.8%.
$M_w = 7.6\times10^4$; $M_w/M_n \approx 1.6$.[53]
HM-pectin
DE=70.2%.
$M_w = 1.1\times10^5$; $M_w/M_n \approx 1.6$.[53]



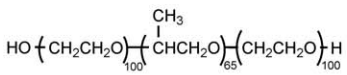
Aginate
G/M=0.75.
$M_w = 1.5\times10^5$.
HM-4C_8-alginate: x=4; m=8.
HM-20C_8-alginate: x=20; m=8.
HM-27C_8-alginate: x=27; m=8.



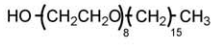
HEC
$MS_{EO} = 2.5$.
$M_w = 4.0\times10^5$; $M_w/M_n \approx 7$.[54]
HM-2C_8-HEC: x=2; m=8.
HM-2C_{12}-HEC: x=2; m=12.
HM-2C_{16}-HEC: x=2; m=16.
HM-1C_{16}-HEC: x=1; m=16.



$EHEC_{1.9}$
DS_{ethyl}=1.9; MS_{EO}=1.3.
$M_n \approx 8\times10^4$; $M_w/M_n \approx 2$.
$EHEC_{0.8}$
DS_{ethyl}=0.8; MS_{EO}=1.0.
$M_w \approx 1\times10^5$.
HM0-0.7C_{12}-$EHEC_{0.8}$: x=0.7; m=12/14; y=0.
HM4-0.7C_{12}-$EHEC_{0.8}$: x=0.7; m=12/14; y=4.



Pluronic F127
$M_n = 1.3\times10^4$.



Octaethylene glycol monohexadecyl ether
$M_n = 594.86$.

Figure 1. Some characteristic data for the polymers, a schematic representation of the repeating units of the polysaccharides, and the structure of pluronic and the C_{16} surfactant. For the HM polymers, *x* denotes the percentage of anhydroglucose units, which carry a hydrophobic group, and m is the length of the hydrophobic alkyl chain. DDA is the degree of deacetylation, MS_{EO} is the average number of hydroxyethyl groups per anhydroglucose unit, DS_{ethyl} is the average number of ethyl groups per anhydroglucose unit, DE is the degree of esterification, G/M is the guluronic acid to mannuronic acid ratio, M_n is the number average molecular weight, M_w is the weight average molecular weight, and M_w/M_n is the polydispersity. Unless otherwise stated, the specifications are according to the manufacturer.

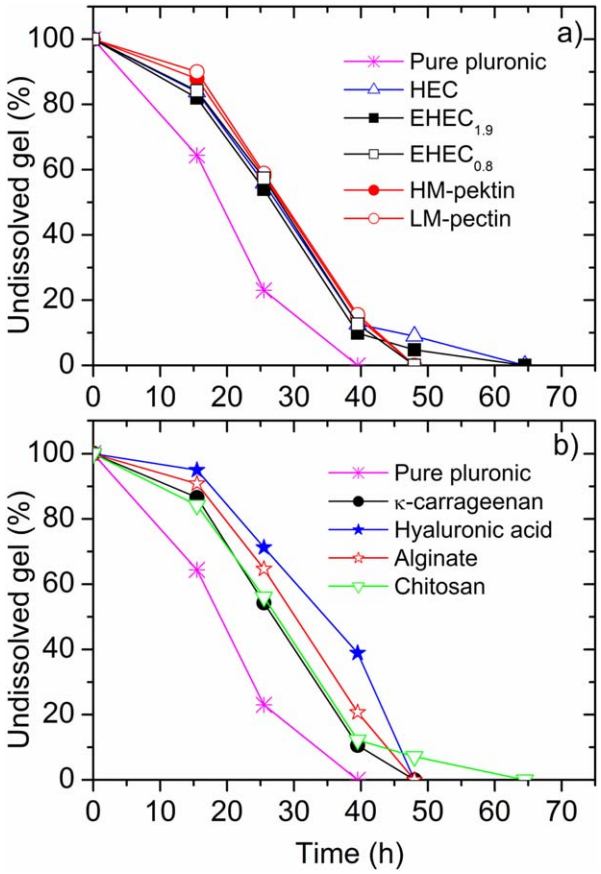


Figure 2. Dissolution at 37°C of 17.5 wt % pluronic gels containing 0.5 wt % of the indicated polysaccharides. The data is divided into two plots (a, b) for clarity. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

polysaccharides addition to the pluronic gel slowed down the dissolution time. A decreased hydrophilic nature of the added polymer and a high molecular weight has been suggested to promote the stabilization of pluronic gels.[41] The polysaccharides used in the present study generally have a much higher molecular weight than pluronic (see Figure 1), and they can thereby provide a network structure that to some extent stabilizes the gels against dissolution. In addition, the hydrophobicity of the polymers should contribute to the stabilization of the gels. At shorter times, the gel containing hyaluronic acid dissolved slower than the other samples. This might be due to its very high molecular weight ($M_w = 1.9 \times 10^6$), which could improve the stabilization of the gels. However, like most of the gels containing polysaccharides, the gels were totally dissolved after 48 h.

The gels containing $EHEC_{1.9}$, chitosan, or HEC took a little longer to dissolve completely than the other samples. Interestingly, this effect only showed up towards the end of the dissolution process, while the profile at shorter times was identical to the other samples. Since these three samples prolonged the time it took before the gels were completely dissolved, identification of differences from the other polysaccharides could be helpful for finding a method to further extend the dissolution times. A schematic representation of the repeating units of the polysaccharides and some of their characteristics are displayed in Figure 1. The main difference between $EHEC_{1.9}$ and $EHEC_{0.8}$ is that the value of DS_{ethyl} for $EHEC_{1.9}$ is more than doubled compared with $EHEC_{0.8}$. The addition of more ethyl groups would make the polymer more hydrophobic. This suggests that increased hydrophobicity of the polysaccharide may prolong the time it takes before the polysaccharide-containing pluronic gels dissolve completely. The hydroxyethyl groups which are present in HEC are also slightly hydrophobic, and might also contribute in the same way. Chitosan, which has a pK_a value of about 6.3–6.5,[50] is neutralized in the PBS (pH = 7.4) used for making the gels. Since chitosan is poorly dissolvable in water above its pK_a value, we have used a chitosan chloride that improves water solubility. However, even though it is possible to dissolve the chitosan sample, it will still be relatively hydrophobic. Interestingly, in a study by Ur-Rehman et al., chitosan was found to speed up the dissolution of pluronic gels.[45] However, the molecular weight of chitosan employed in our study was much higher, suggesting that the molecular weight of the polymer plays an important role in the stabilization process. The remaining polysaccharides κ-carrageenan, hyaluronic acid, alginate, and the pectins are all quite hydrophilic at the current conditions, again in agreement with the hypothesis that a higher hydrophobicity

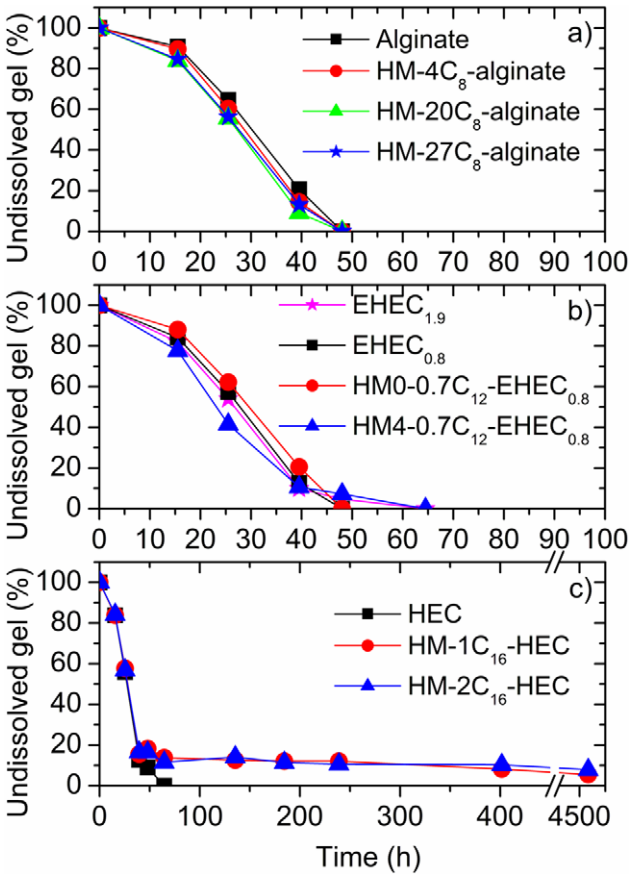


Figure 3. Dissolution at 37°C of 17.5 wt % pluronic gels containing 0.5 wt % of (a) alginate of different hydrophobicity, (b) EHEC of different hydrophobicity, and (c) HEC of different hydrophobicity. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

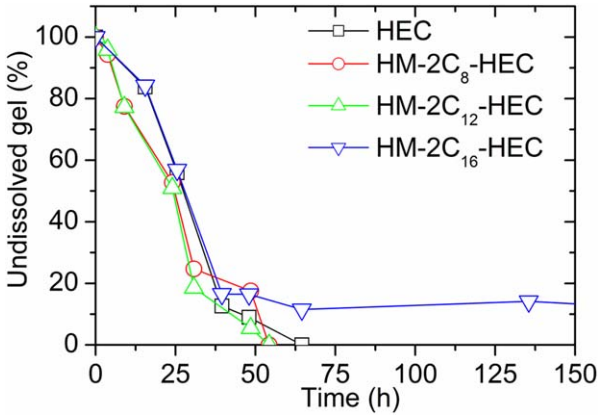


Figure 4. Dissolution at 37°C of 17.5 wt % pluronic gels containing 0.5 wt % HM-HEC with about 2 mol % hydrophobic groups of different lengths. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of the polysaccharides postpones the time it takes before the entire gel sample has dissolved. This suggests that interactions between hydrophobic domains on the polysaccharides and the pluronic gels can help to stabilize the gels from dissolving.

While κ-carrageenan, chitosan, hyaluronic acid, pectin and alginate all carry charges, HEC and EHEC are neutral polymers. As can be seen from Figure 2, there does not seem to be any correlation between the charged or neutral nature of the polysaccharides and the dissolution time of the gels.

To further test this hypothesis, HM analogues of some of the polysaccharides were tested. As can be seen from Figure 3(a), attaching various amounts of amide-linked C8 groups to alginate do not prolong the dissolution time of the gels compared to the pure alginate sample. We also tried to use alginates with an even higher amount of attached hydrophobic groups. However, these samples did not dissolve properly in the pluronic/PBS solutions.

Figure 3(b) show that attaching 0.73 mol % of a 70/30 mixture of C12 and C14 groups to $EHEC_{0.8}$ (HM0-0.7C_{12}-$EHEC_{0.8}$) did not significantly prolong the time it took before the gel was completely dissolved. When a spacer of 4 EO groups was inserted between the hydrophobic groups and the polymer backbone (HM4-0.7C_{12}-$EHEC_{0.8}$) the weight of the remaining gels actually decreased a little faster at shorter times, but the time it took the gel to dissolve completely increased, reminiscent of what was observed for the $EHEC_{1.9}$ sample. A possibility is that the pluronic gels are stabilized by hydrophobic groups that penetrate into the hydrophobic PPO core of the micelles. Since the hydrophobic groups are attached to a polymer with a relatively high molecular weight, the polysaccharide could act as a scaffold binding together and stabilizing the pluronic gels. This hypothesis would require the hydrophobic substituents to be of a certain length, and may explain why the HM4-0.7C_{12}-$EHEC_{0.8}$ sample containing a spacer group would work better than the HM0-0.7C_{12}-$EHEC_{0.8}$ without a spacer.

Interestingly, the addition of 1 or 2 mol % C_{16} groups to HEC (HM-1C_{16}-HEC and HM-2C_{16}-HEC), keeps the gels from dissolving completely for more than 6 months [see Figure 3(c)]. The number of hydrophobic groups does not seem to have a significant effect on the dissolution rate. This indicates that attaching very long hydrophobic groups to the polysaccharide can stabilize a significant part of the pluronic gels for a very long time. This is supporting the hypothesis that the hydrophobic groups need to be long enough to penetrate into the core of the pluronic micelles, and together with the polymer backbone form bridges between the cores.

Although a part of the gels remains undissolved even after 6 months, a significant amount of the gels dissolves relatively fast. This might be explained by the following scenario: Pluronic micelles that are not attached to the HM-HEC hydrophobic groups can relatively easily escape out from the gel network. HM-HEC will to a higher extent be prevented from escaping since it penetrates longer into the gel network. In addition, pluronic micelles can be attached to more than one hydrophobic group belonging to different HM-HEC chains, thereby providing cross-linking points, which gives an interconnected network that anchors the HM-HEC chains in the gel. Accordingly, the gel initially dissolves quite fast due to the release of pluronic micelles that are not attached to any hydrophobic groups. Although some of the HM-HEC probably also dissolves, this process is much slower. When most of the nonattached pluronic micelles has dissolved, the remaining interconnected network consist of HM-HEC chains stabilized by pluronic micelles that act as crosslinking points between the hydrophobic pendant groups. The resulting gel is quite stable against further dissolution, causing the observed two-step behaviour in the dissolution profiles in Figure 3(c). To confirm that the improved stabilization is due to the length of the hydrophobic groups and not caused by some properties of HEC itself, HM HEC with shorter hydrophobic groups were also prepared. As can be seen from Figure 4, the addition of 2 mol % C_8 or C_{12} groups to HEC did not prevent the gels from dissolving at long times, while the same amount of C_{16} groups prevented the dissolution of the gels for a long period of time. It is reasonable to assume

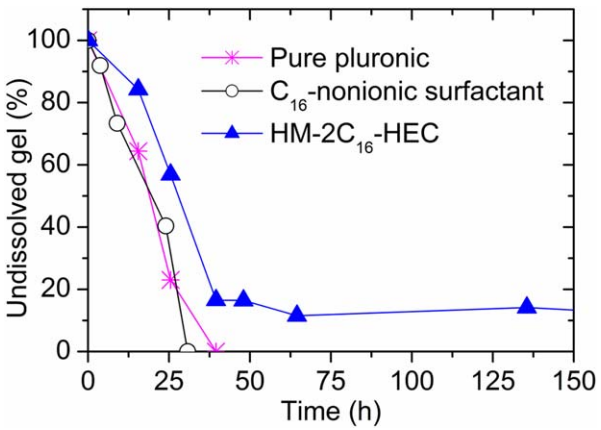


Figure 5. Dissolution at 37°C of 17.5 wt % pluronic gels compared to the same gels containing 0.5 wt % of a C_{16} nonionic surfactant (octaethyleneglycol monohexadecyl ether) or 0.5 wt % of HM-2C_{16}-HEC (which contains 2 mol % C16 groups). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

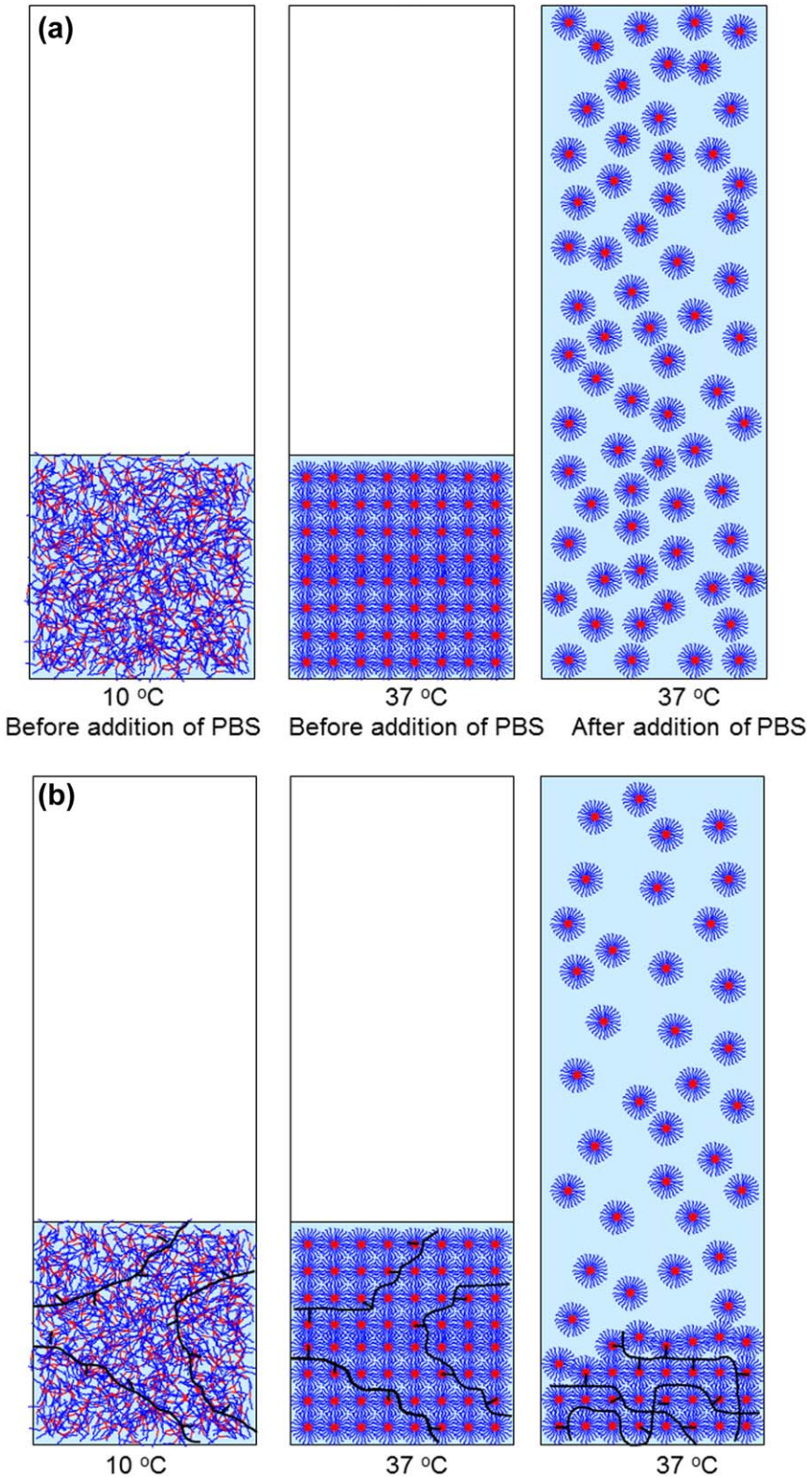


Figure 6. Schematic sketch of the gelation and dissolution/stabilization of pluronic gels. (a) In the absence of a stabilizing polysaccharide. (b) In the presence of a stabilizing polysaccharide containing relatively long hydrophobic groups (see text for details). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that the gels are stabilized by a combination of the network formation of the polysaccharide of a relatively high molecular weight combined with the interaction between the long C_{16} chains and the core of the pluronic micelles. The last point of the curves (where the gel is completely dissolved) appears earlier for HM-2C_8-HEC and HM-2C_{12}-HEC than for the unmodified HEC sample. This is probably just caused by the differences between the times where these points were measured. To make sure that the presence of C_{16} chains alone could not stabilize the pluronic gel network, a C_{16} nonionic surfactant (octaethylene glycol monohexadecyl ether) was also tested. As can be seen from Figure 5, the addition of a C_{16} nonionic surfactant did not slow down the dissolution of the pluronic gels.

A schematic sketch of a possible mechanism for the stabilization of the pluronic gels is shown in Figure 6. At a low temperature (10°C), the pluronic chains are randomly oriented in the solution. At higher temperatures (37°C), the PPO-block becomes more hydrophobic, and the pluronic is organized into micelles. If the pluronic concentration is sufficiently high, the micelles form a close packed cubic structure, which causes the formation of a gel. When a solvent (PBS) is added to the top of the gel, the micelles start to diffuse out into the solvent. In the absence of a stabilizing polymer, the gel will eventually dissolve completely [Figure 6(a)]. In the presence of a polysaccharide containing hydrophobic groups of a sufficient length, the hydrophobic groups can penetrate into the hydrophobic PPO core of the pluronic micelles. The micelles that are bound to the polysaccharide are thus prevented from disengaging out of the gel network, and the gels are stabilized against dissolving completely [Figure 6(b)].

CONCLUSIONS

All the tested polysaccharides slow down the dissolution of the pluronic gels, but the gels are still fully dissolved after 2–3 days. The modification of the polysaccharides by attaching relatively short hydrophobic chains (C_8 or C_{12}) does not affect the dissolution rates very much, even at high modification degrees. However, modifying the polysaccharides with C_{16} groups prevents dissolution of a specific fraction of the gels over a long time (> 6 months). C_{16}-surfactants do not have the same effect. Accordingly, to stabilize the pluronic gels we need a polymer with a sufficiently high molecular weight to make a network, which in addition also contain long hydrophobic chains attached to the polymer backbone.

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