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Original stimuli-sensitive polysaccharide derivatives/N-isopropylacrylamide hydrogels. Role of polysaccharide backbone

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ABSTRACT

This article compares the properties of a novel class of unsaturated Xanthan and Gellan derivatives/N-isopropylacrylamide stimuli-responsive hydrogels synthesized by free radical polymerization. Xanthan and Gellan Gum were partially functionalized by esterification with maleic anhydride under various conditions. By copolymerization of these maleate polysaccharides with a N-isopropylacrylamide known temperature sensitive precursor, water-swollen hydrogels with interpenetrating polymer networks (IPN) were obtained. The hydrogels were characterized for their temperature and pH-responsive behaviour by equilibrium swelling experiments and differential scanning calorimetry. The investigation of these materials also includes solid-state ^{13}CP/MAS NMR and elemental analysis of the nitrogen content. Morphology was visualized by scanning electron microscopy. Depending upon composition and the nature of the base-polysaccharide, the hydrogels showed different response rates to the external changes of temperature as well as pH. By changing the feed composition ratio of precursors and crosslinking agent (β-cyclodextrin acrylate or N,N′-methylenebisacrylamide respectively) the phase transition temperature (lower critical solution temperature) could also be adjusted near to the body temperature for biomedical and biotechnological applications. The role of the rigidity and the charge density of the polysaccharide chain, its ability to form hydrogen bonding on these properties are more particularly considered.

1. Introduction

Medical applications need more and more specialized materials in order to reach their objectives such as the increase of therapeutic effects, the reduction of noxious effects and the development of new therapeutic strategies. In this domain controlled released systems allowing a drug controlled and targeted escape were widely studied (Qiu & Bae, 2006). Hydrogels are a promising approach in numerous pharmaceutic and biomedical applications because of their capacity to retain high water contents, their ease of handling etc. even if biocompatibility is an important point which, in some cases, has to be overcome (Hoffman, 2002; Peppas, Bures, Leobandung, & Ichikawa, 2000).

Polysaccharides are ideal candidates because they are biocompatible, biodegradable, hydrophilic, and some of them may form hydrogels or may be chemically modified to gain these properties. To modulate the controlled release properties of hydrogels a new way may consist in obtaining semi- or full-interpenetrated networks (semi- or full-IPN). In this context, hydrogels obtained by combination of hydrophilic and hydrophobic polymers may lead to new materials. The hydrophobic polymer, generally a synthetic one, may act as the drug carrier whereas the hydrophilic polysaccharide provides biological interactions. Amongst these polysaccharides, Gellan and Xanthan gums were widely used in the food, cosmetics formulations, pharmaceutic and biomedical industries. Gellan gum (GG) is an anionic deacetylated exocellular polysaccharide secreted by *Pseudomonas elodea* with a tetrasaccharide repeating unit of one α-L-rhamnose (Rhap), one β-D-glucuronic acid (GlcpA) and two β-D-glucoses (Glcp) (Fig. 1a) as proposed by Jansson, Lindberg, and Sandford (1983). Xanthan Gum, produced by *Xanthomonas campestris* (Kang & Pettit, 1993), has a cellulosic backbone of D-glucose linked in β-1,4 positions. For every alternate glucose unit there is a side chain consisting of β-D mannose-(1,4)-β-D-glucuronic acid-(1,2)-α-D-mannose (Melton, Mindt, Rees, & Sanderson, 1976). The terminal mannose may carry pyruvate residues linked to the positions 4 and 6. The internal mannose unit

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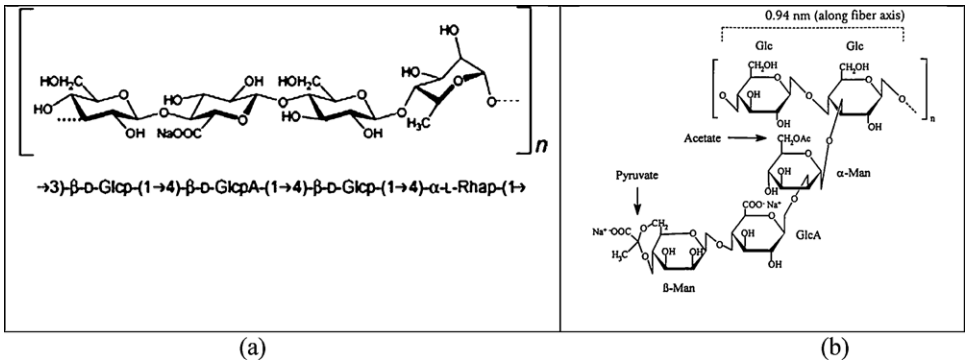


Fig. 1. Repeating unit of deacetylated Gellan gum sodium salt (a) and Xanthan gum (b).

is partially acetylated at C-6 (Smith, Symes, Lawson, & Morris, 1981; Sutherland, 1981) (Fig. 1b).

Aqueous solutions of Gellan gum and Xanthan form physical gels. In the presence of cations (Crescenzi, Dentini, & Coviello, 1990), the mechanism of gelation of Gellan gum involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water (Milas & Rinaudo, 1996; Ogawa, Matsuzawa, & Iwahashi, 2002; Takahashi et al., 2004). In pharmaceutical and biomedical industries, its gel forming properties in the presence of multivalent cations have been exploited for controlled release of drugs (Balasubramaniam, Thilek Kumar, Pandit, & Kant, 2004; Mukai-Correa, Prata, Alvim, & Grosso, 2004; Ottoboni, Ronald, & Stanley, 2000). The mechanical stability of the ionically crosslinked gel is provided by the combination of multivalent, generally divalent, cations, such as calcium (Crescenzi, Dentini, & Dea, 1987). For the applications as drug release system in the physiological environment, extracellular concentrations of monovalent cations (such as sodium ions) exceed the concentration of divalent ones (such as calcium). Therefore, the ionic gels tend to lose their mechanic stability over the long term due to diffusion, leading to exchange of divalent cations for monovalent ones in the physiological fluid (Crescenzi, Dentini, & Dea, 1987; LeRoux, Guilak, & Setton, 1999; Stokke, Christensen, & Smidsrod, 1998); so they are not interesting as drug controlled release carriers. To avoid these problems, chemical modifications of Gellan gum and Xanthan were proposed (Hamcerencu, Desbrieres, Popa, Khoukh, & Riess, 2007; Hamcerencu, Desbrieres, Khoukh, Popa, & Riess, 2008). As examples, a polysaccharide carrying unsaturated substituents, which provides multiple centres for grafting/crosslinking, could result in stronger gels and more tightly controlled mechanical and degradation behaviour than those physically/ionically crosslinked gels (Berger, Reist, Mayer, Felt, & Peppas, 2004; Coutinho et al., 2010; Matricardi, Cencetti, Ria, Alhaique, & Coviello, 2009; Stokke et al., 1998). If the connecting grafted chains are linked to the polysaccharide backbone, it can be assumed that those of acrylic type are hydrolysis resistant in physiological environment in contrast to the maleate semi-esters. Nevertheless maleate semi-esters could have the advantage for the design of hydrogels which crosslink density decreases with time and thus becoming more readily bioresorbable.

Polysaccharide hydrogels are mainly produced by several different approaches. Chemical gels cross-linked by covalent bonds can be obtained either by employing crosslinking agents such as epichlorohydrin or carbodiimide or by grafting/crosslinking with acrylic monomers. Epichlorohydrin, as many other crosslinking agents, is known to be cytotoxic. Moreover, short crosslinking bridges introduced by such a crosslinking agent between polysaccharide backbones would lead to a final product having a high rigidity in the dry state. The length of crosslinking bridges can be adjusted by grafting/crosslinking of polysaccharide with monomers, amongst which acrylics occupy a significant position (Hamcerencu, Desbrieres, Popa, & Riess, 2009). By this way, longer and/or temperature or pH stimulating bridges are introduced between polysaccharide chains. A less rigid structure and an easier control of network density, as a function of number of double bonds introduced on the polysaccharide backbone, may thus be attained.

Both Xanthan and Gellan gum present a polyelectrolyte character due to the presence of carboxylic groups within the macromolecular chain. They differ essentially by the chain stiffness, Xanthan being stiffer as described by its persistance length, the Xanthan one being equal to 100 nm at infinite ionic strength whereas for Gellan it is 72 nm (Milas, Shi, & Rinaudo, 1990). In the same time, B stiffness parameter differs for the two polysaccharides, 0.005 for Xanthan whereas 0.05 for Gellan (Jampen, Britt, & Tung, 2000; Smidsrod & Haug, 1971). Moreover the charge density is higher for Xanthan compared with Gellan. Both of them present a helix-coil transition depending on the salt concentration and the temperature (Mazen, Milas, & Rinaudo, 1999; Milas & Rinaudo, 1986; Milas, Rinaudo, Duplessix, Borsali, & Lindner, 1995).

Amongst the thermosensitive polymers, poly(N-isopropylacrylamide) PNIPAAm is the most investigated one for biomedical applications, exhibiting a LCST close to body temperature. Clinical applications of NIPAAm-based thermosensitive hydrogels have limitations since they are not biodegradable but have been evaluated as drug release carriers consisting in semi- or full-IPN of PNIPAAm and dextran, chitosan and Xanthan. Our intention was to associate a polysaccharide with NIPAm monomer to obtain hydrogels presenting in the same time thermosensitive properties.

One of the limitations of this class of hydrogels is their limiting capacity to load poorly water-soluble drugs. A new approach to solve this problem involves the incorporation of cyclodextrins into the polymer network. In this way, the formulation of hydrophobic drugs in hydrophilic media became possible by combining the complexation properties of cyclodextrins with the versatility of the hydrogels (Hamcerencu et al., 2009).

Compared to other approaches that require the use of organic solvents and sophisticated carriers, the preparation of cyclodextrin hydrogels involves simple technological processes for the elaboration of more biocompatible and stable materials. For this purpose, beta-cyclodextrin, first modified with unsaturated binding functionalities as described elsewhere (Hamcerencu, Popa, Riess, Ritter, & Alupei, 2005), was simultaneously used as a hydrophobic drug carrier and crosslinking agent.

To the best of our knowledge it is the first time that these modified Xanthan or Gellan-based networks are compared. In the present study, their morphology, temperature, and pH-responsive behaviour will be examined. Their swelling properties and the role

Table 1
Composition of initial reaction mixtures used for the preparation of hydrogels based on MA-P/PNIPAm crosslinked with BIS (a) and A-CD (b).

Sample code	MA-P/NIPAm ratio (%, wt/wt)	
	MA-P	NIPAm
(a)		
NB0	–	100
GNB1	10	90
GNB2	25	75
GNB3	50	50
XNB1	10	90
XNB2	25	75
XNB3	50	50
(b)		
NC0	–	100
GNC1	10	90
GNC2	25	75
XNC1	10	90
XNC2	25	75

The concentration of polysaccharide maleate is 2.5% (wt/v) for all the hydrogels. The concentration of the crosslinking agent (BIS or A-CD) is 3% (wt/wt) with respect to comonomers mixture. Initiator: APS (3.5%, wt/wt, reported to comonomers mixture); activator: TEMED (1.1 mol/1 mol APS).

of the crosslinking agent and/or the polysaccharide backbone on the hydrogel properties will be discussed keeping in mind that this comparative study of the hydrogel characteristics, as a function of the various formulation parameters, was carried out in view of their practical applications as thermo- and pH-responsive biomedical materials. More accurately ophthalmic applications will be focused.

2. Experimental

2.1. Materials

Precursor Gellan was obtained from CP Kelko. Xanthan gum (from *X. campestris*) was obtained from BioChemika (degree of substitution per side chain of 0.73 and 0.75 for acetate and pyruvate groups, respectively, as determined by proton NMR (Hamcerencu et al., 2007). Maleate Gellan (MA-G) (with a degree of substitution, DS, of 15.4%), maleate Xanthan (MA-X) (DS of 10.8%) and β-cyclodextrin acrylate (A-CD) (with a DS ≅ 3) were synthesized and characterized according to our previous published procedures (Hamcerencu et al., 2007, 2008). Considering Xanthan the degree of substitution DS of 10.8% with respect to the total amount of OH groups per repeating unit corresponds to around 50% with respect to the 2 primary OH groups of the repeating unit; in other words each repeating unit of Xanthan contains therefore on the average 1 maleic double bond and Gellan, 1.5 maleic double bond per repeating unit. N-isopropylacrylamide (NIPAm), N,N′-methylene bisacrylamide (BIS), N,N,N′,N′-tetramethylenediamine (TEMED), were purchased from Sigma–Aldrich and used as received. Ammonium persulphate (APS) was obtained from Merck.

2.2. Preparation of hydrogels

Hydrogels were synthesized by a free-radical grafting-crosslinking reaction of unsaturated esters of polysaccharides (more generally called in this paper MA-P) with N-isopropylacrylamide, using APS as initiator activated by TEMED and BIS or A-CD as crosslinking agents. In the present formulations the polysaccharide concentration was kept constant and the comonomers ratio was varied. The compositions of initial reaction mixtures used for elaboration of hydrogels are given in Table 1. In the sample code of the hydrogel formulations **G** represents Gellan maleate, MA-G, **X**: Xanthan maleate, MA-X, **N**: NIPAm, **B**: BIS, **C**: A-CD. For instance, GNB1 corresponds to a formulation including Gellan maleate, N-isopropylacrylamide and N,N′-methylene bisacrylamide.

The mixture of comonomers (MA-G or MA-X and NIPAm) and the crosslinking agent are dissolved in bi-distilled water, under magnetic stirring, during 24 h. The initiator (APS, 3.5%, wt/wt, with respect to the comonomers mixture) was then added and stirring was pursued during one hour. This solution was transferred in a container fitted with a septum equipped screwtop and degassed with nitrogen during 20 min to eliminate oxygen from the reaction medium. The activator-TEMED (1.1 mol/1 mol of APS) was then added using a syringe. The reaction was carried out at room temperature (20 °C; $T <$ LCST of NIPAm and below the coil-helix transition temperature of the polysaccharide) during 24 h. As a comparison, hydrogels based on NIPAm only were synthesized in the same conditions. Directly after synthesis and without preliminary drying to avoid specific aggregation, obtained hydrogels were immersed during 14 days in distilled water to eliminate chemicals which have not reacted (water being changed every 6 h), and finally in acetone during one hour before drying at room temperature (7 days) and then under vacuum (0.1 atm, 4 days).

2.3. Determination of the hydrogel composition

The composition of hydrogels based on polysaccharides and PNIPAm was determined from the measure of nitrogen content using Kjeldhal method.

Hydrogels were characterized by their structure (solid NMR), composition (nitrogen element analysis), morphology (scanning electron microscopy – SEM), thermal properties (DSC), swelling characteristics in water and other aqueous solutions (swelling kinetics, maximal swelling degree). All these characterizations were carried out on purified hydrogels.

2.4. Physico-chemical properties

2.4.1. ^{13}C CP/MAS NMR spectroscopy

Samples, approximately 300 mg, as fine powder were introduced in the rotor which diameter is 7 mm. Cross polarization Magic Angle Spinning (CP/MAS) NMR spectra were recorded at a nominal frequency of 100.6 MHz on a Bruker Avance instrument operating at 400.13 MHz equipped with a PH MASVTN400SB BL7 probe twofold tuned on ^{1}H et ^{13}C nuclei. Analysis was performed at magic angle spinning (54°44″) and at a frequency of 6 kHz ± 1 Hz. Polarization transfer was obtained with a contact time of 3.8 ms. Spectra were recorded at 25 °C with a recycling time of 5 s and 12,000 scans. The CPSELTICS impulsion sequence used was supplied by Bruker.

2.4.2. Differential scanning calorimetry (DSC)

To determine the thermal behaviour of polysaccharide/PNIPAm based hydrogels, more especially their LCST and the influence of external stimuli, the hydrogel samples were immersed at room temperature during 24 h in either bi-distilled water or in solutions with different pH or ionic strength (NaCl solutions). After elimination of excess liquid, the DSC analysis was performed in a temperature range from 10 to 50 °C, with a 2 deg/min temperature ramp under nitrogen atmosphere (50 mL/min) using a Q100 calorimeter (TA Instruments, USA). Each sample was submitted to three successive heating–cooling cycles with a 5 min isotherm previously to each cycle.

2.4.3. Swelling studies

Swelling studies of hydrogels were carried out according to the modified Dogadkin method (Alupei, Popa, Hamcerencu, & Abadie, 2002) for all systems and moreover by thermogravimetry when salt containing solutions were concerned (Hamcerencu et al., 2009).

The swelling ratio was determined from relation (1) (Paradossi, Chiessi, Cavalieri, Moscone, & Crescenzi, 1998):

$$Q_m\ (\%) = \frac{m - m_0}{m_0} \times 100 = \frac{m_1}{m_0} \times 100 \quad (1)$$

where Q_m is the mass swelling degree (%); m_0, the mass of dry hydrogel (g); m the sample mass after swelling (g); m_1 the weight of swelling agent retained by sample (g).

2.4.4. Morphology of hydrogels

Scanning electron microscopy (SEM) was used to study the morphology of hydrogels using SEM Philips 525 M equipped with a chemical analysis system (EDX), working at 20 kV respectively. At the maximal swelling degree the samples are freeze-dried in liquid nitrogen before gold plating. All characterizations were carried out on purified hydrogels.

3. Results and discussion

3.1. Synthesis of hydrogels

Thermosensitive hydrogels are elaborated by crosslinking of the functionalized polysaccharide acting as macromonomer in a grafting-copolymerization reaction of NIPAm. As a general rule, this monomer was grafted to the polysaccharidic chain forming macroradicals which, afterwards, through specific termination reactions, mainly by recombination, lead to grafted or crosslinked structures (Morimoto, Qiu, Winnik, & Akiyoshi, 2008; Tang, Hua, Cheng, Jiang, & Zhu, 2008). Referring to these termination reactions, the PNIPAm chain will constitute the bridge between two polysaccharidic chains.

The adjustment of hydrogel crosslinking degree, hence the swelling degree in water as well, may be achieved with the bifunctional crosslinking agent, BIS. It is obvious that the structure of the final products resulting from the ternary "macromonomer–NIPAm–BIS" system is extremely complex, implying on one hand the polysaccharidic network crosslinked with NIPAm bridges and BIS, on the other hand the NIPAm network directly crosslinked with BIS, both networks being formed simultaneously. In the absence of BIS, it is also obvious that even non-crosslinked NIPAm homopolymer could be formed. However, this homopolymer is eliminated from the gel during the purification step. It is important to mention that polysaccharide maleate itself may also react with BIS. In this case hydrogel-like structures of low crosslinking density were obtained, these materials being more similar to viscous solutions rather than gels. Due to their high swelling ratio, they are practically unusable for the aimed applications. A general representation of the crosslinked structures is given in Scheme 1.

Polysaccharide maleates and NIPAm based hydrogels may be considered as semi-interpenetrated networks whereas those implying BIS as the crosslinking agent are full-interpenetrated networks.

If these types of hydrogels are suitable for the incorporation of water-soluble drugs, another objective was to obtain a hydrogel able to include either water soluble or liposoluble drugs. As it is well known that cyclodextrins, due to their peculiar structure, have the ability to include hydrophobic compounds within their hydrophobic cavity (Loftsson, Kristmundsdottir, Ingvarsdottir, Olafsdottir, & Baldvinsdottir, 1992), a process was developed in order to chemically bind such a cyclic oligosaccharide (β-cyclodextrin) within polysaccharide derivative and NIPAm based networks. The chosen way was the functionalization of the oligosaccharide by introducing unsaturated substituents, as the formation of cyclodextrin acrylates. The reaction was not quite selective, and by using an excess of acryloyle chloride as esterifying agent a mixture of di- and polyfunctional derivatives was obtained (Hamcerencu et al., 2005), designated in the following by "β-cyclodextrin acrylate" or A-CD. The synthesis of structures including the cyclodextrin was performed by a ternary copolymerization reaction in which the participant monomers were polysaccharide maleate, NIPAm and "cyclodextrin acrylate" (A-CD). The high degree of functionalization of the cyclodextrin (DS ≅ 3) allows the A-CD to act directly as a crosslinking agent leading to structures as described in Scheme 2. As a consequence, the use of BIS was not necessary for this hydrogel series. The chemical reactions involved in the preparation of the hydrogels are the same whatever the crosslinking agent: a free radical grafting-polymerization reaction of NIPAm using persulphate ions used as initiators and the presence of OH° radicals. The only difference comes from the functionality of the crosslinking agent, 2 for BIS and 3 for cyclodextrine acrylate which is illustrated in Schemes 1 and 2.

According to the nature of the crosslinking agent (BIS or A-CD) and their different functionalities the final structures of the hydrogels will be different as demonstrated by Schemes 1 and 2. As a consequence, different properties will be expected considering swelling, inner morphologies as it will be discussed later. Finally inclusion and release power will be dependent upon these characteristics and hence on the crosslinking agent.

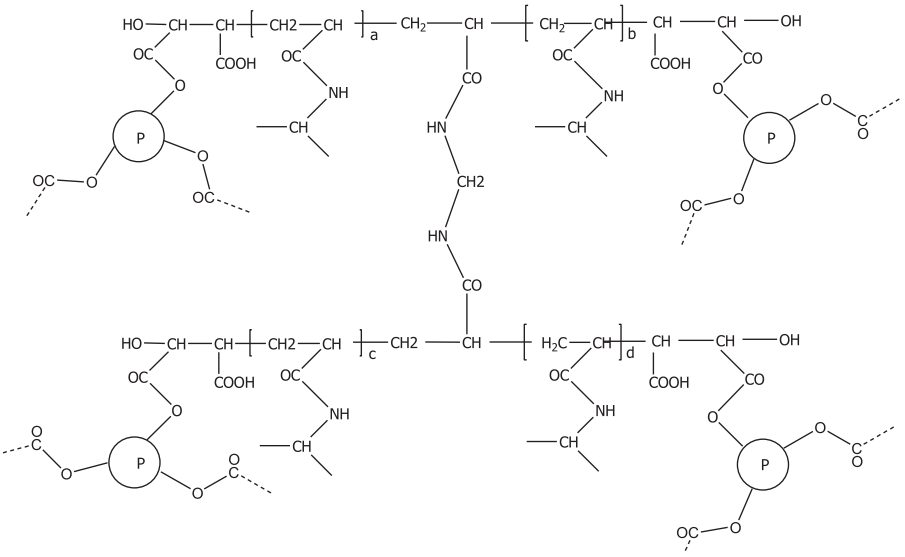
3.2. Composition and structure of hydrogels

The final hydrogels composition, compared to those of the initial comonomer mixture (MA-P, NIPAm), may offer information on the reactivity of the different components during the grafting-crosslinking copolymerization reaction. After purification, the PNIPAm content of the final material was determined by elemental analysis of nitrogen content. The corresponding results are given in Table 2.

It should be mentioned that a simplifying assumption was considered for calculations of the hydrogel composition. NIPAm and BIS are only nitrogen sources within the hydrogel. However as the BIS content was very low, as compared to that of NIPAm one, whatever the hydrogel preparation, its contribution to the nitrogen content was neglected. Hence, NIPAm was considered as the only contribution to the nitrogen content of all samples.

From Table 2 it appears clearly that, for BIS and A-CD crosslinked samples, the hydrogel composition is very close to that of the initial comonomer mixture within the experimental error limits. All comonomers (MA-X or MA-G, NIPAm) and even BIS are integrally incorporated in the hydrogel, indicating that all components of the system are of a high reactivity. The XN1 sample has not a close composition to that of the original mixture. This is explained by the fact that no additional crosslinking agent (BIS or A-CD) was added. As a consequence we have simultaneously NIPAm polymerization and grafting on polysaccharide and a very few crosslinking occurs through PNIPAm grafts. A mixture of hydrogel and PNIPAm homopolymer was observed. This homopolymer may be removed by washing at room temperature. This explains the smaller PNIPAm content in XN1 hydrogel compared to the initial composition. In the same time, this demonstrates the efficiency of PNIPAm segments as crosslinking agents.

The presence of the two comonomers (MA-X or MA-G and NIPAm) within the hydrogel was confirmed by spectroscopic techniques such as FT-IR (not shown) and CP/MAS NMR (Fig. 2). If Gellan based hydrogels are considered, signals at 23 ppm are assigned to Carbon atoms of methyl groups of PNIPAm (peak a). A broad signal around 42 ppm was assigned to methylene (—CH_2—) and methine (—CH<) groups (peak b), carbonyl group being observed at 175.2 ppm (peak c) for NIPAm. A broad signal was observed around

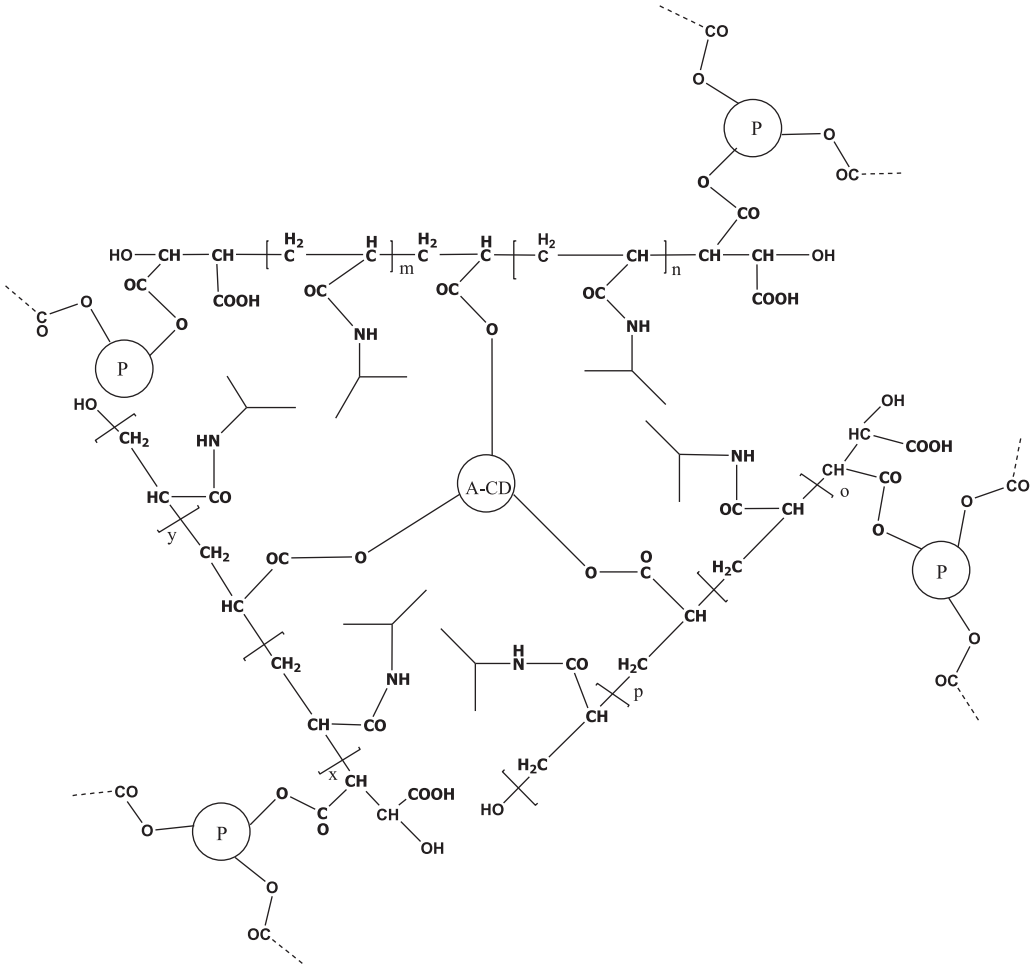


Scheme 1. Structure of a network based on polysaccharide (P) maleate and NIPAm crosslinked with BIS, initiated by free-radical mechanism.

100 ppm when the hydrogels containing polysaccharides were considered, due to anomeric carbon atoms of the polysaccharide (peak d). Signals appearing in the 72–75 ppm region were assigned to other carbon atoms of the Gellan structure (peak e).

3.3. Morphology of hydrogels

Fig. 3 presents SEM photographs for a series of freeze-dried MA-P based hydrogels prepared with BIS or A-CD as crosslinking agent.



Scheme 2. Structure of a network based on polysaccharide (P) maleate and NIPAm crosslinked with "β-cyclodextrin acrylate" (A-CD).

Table 2
Composition of hydrogels based on MA-P and NIPAm, determined from nitrogen elemental analysis.

Sample Code	Initial composition (%, wt/wt)		BIS* (% wt/wt)	N_{th} (%)	N_{exp} (%)	Final composition (%, wt/wt)
	MA-P	NIPAm				NIPAm
NB0	–	100	3	12.5	12.3	99.1[a]
GNB1	10	90	3	11.3	11.5	92.6
GNB2	25	75	3	9.5	9.1	73.7
GNB3	50	50	3	6.5	6.5	52.9
XB1	100	–	3	0.5	0.6[b]	--
XNB1	10	90	3	11.3	11.1	89.5
XNB2	25	75	3	9.5	9.1	73.5
XNB3	50	50	3	6.5	5.9	47.7
XN1	25	75	–	9.3	6.4	51.9
Sample Code	Initial composition (%, wt/wt)		A-CD* (%, wt/wt)	N_{th} (%)	N_{exp} (%)	Final composition (%, wt/wt)
	MA-P	NIPAm				NIPAm
NC0	–	100	3	12.4	12.1	97.8[a]
GNC1	10	90	3	10.8	10.4	87.5
GNC2	25	75	3	9.0	9.2	75.6
XNC1	10	90	3	10.8	10.5	87.4
XNC2	25	75	3	9.0	9.3	75.5

* With respect to comonomer mixture; [a]BIS + NIPAm; [b]referring to BIS.

All the samples appear as homogeneous structures without any apparent macroscopic phase separation of the polymeric constituents. Gellan based hydrogels present a more porous morphology compared with Xanthan based hydrogels (GNB2 and XNB2 samples). GNB1 hydrogel has pores with an approximate diameter from 1 to 5 μm. It may be considered as a mesogel. As observed with Xanthan based hydrogels, increasing the MA-G content reduces the porosity which becomes very compact in the 25–50% of MA-G (GNB3). Moreover a fibrillar-like structure was observed for GNB3 hydrogels. Using cyclodextrin acrylate as a crosslinking agent leads, also, to a macrogel morphology with similar pore diameters than Xanthan based hydrogels. The pore size is larger when cyclodextrin acrylate was used compared with BIS (GNC3 compared to GNB3).

3.4. Phase transition temperature (LCST)

PNIPAm exhibits a lower critical-solution temperature at 32 °C above which the polymer is insoluble, and the transition from a homogeneous mixture to phase separation is abrupt, within 1–2 degrees (Ju et al., 2006). The formation of hydrogels by association of PNIPAm with other polymers opens perspectives to obtain polymer-drug systems able to release in a controlled manner the active component. In fact, at a temperature close to LCST the collapse of the hydrogel leads to the drug release from the network in the medium. Ideal conditions may be that phase transition occurs at a temperature slightly higher than physiological temperature (37 °C) so that the drug may be

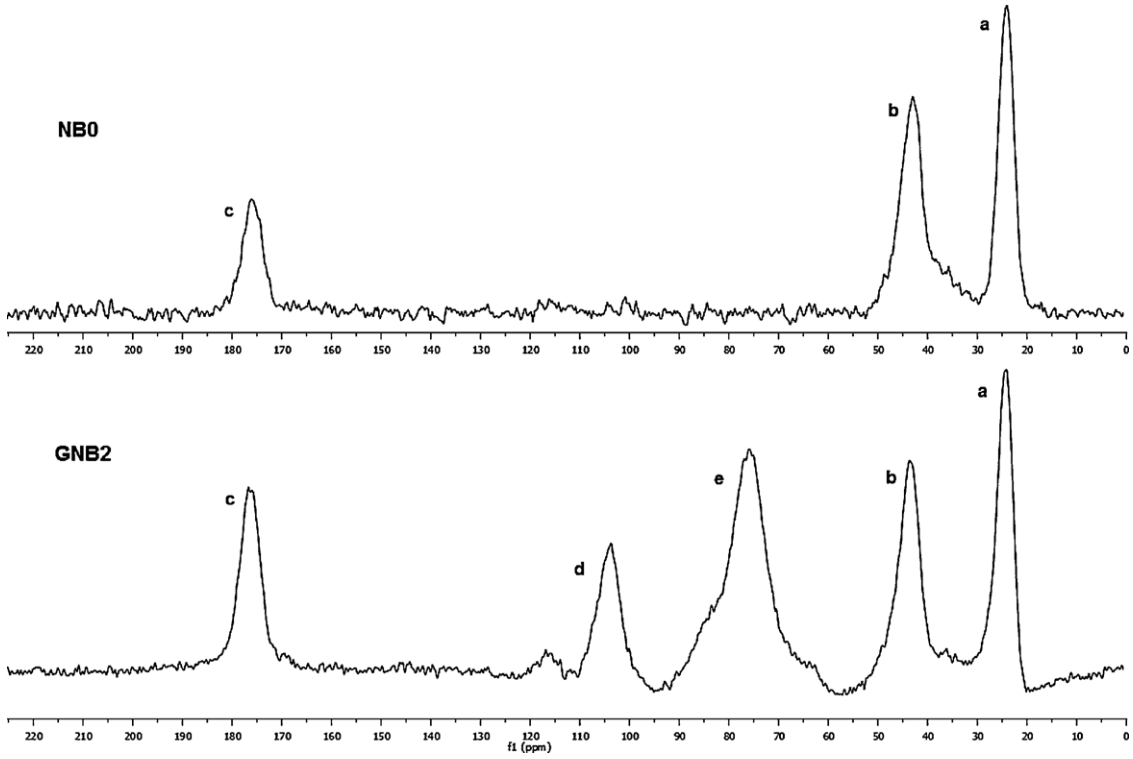


Fig. 2. CP/MAS NMR spectra of cross-linked PNIPAm (NB0) and cross-linked-grafted MA-Gellan–PNIPAm (GNB2).

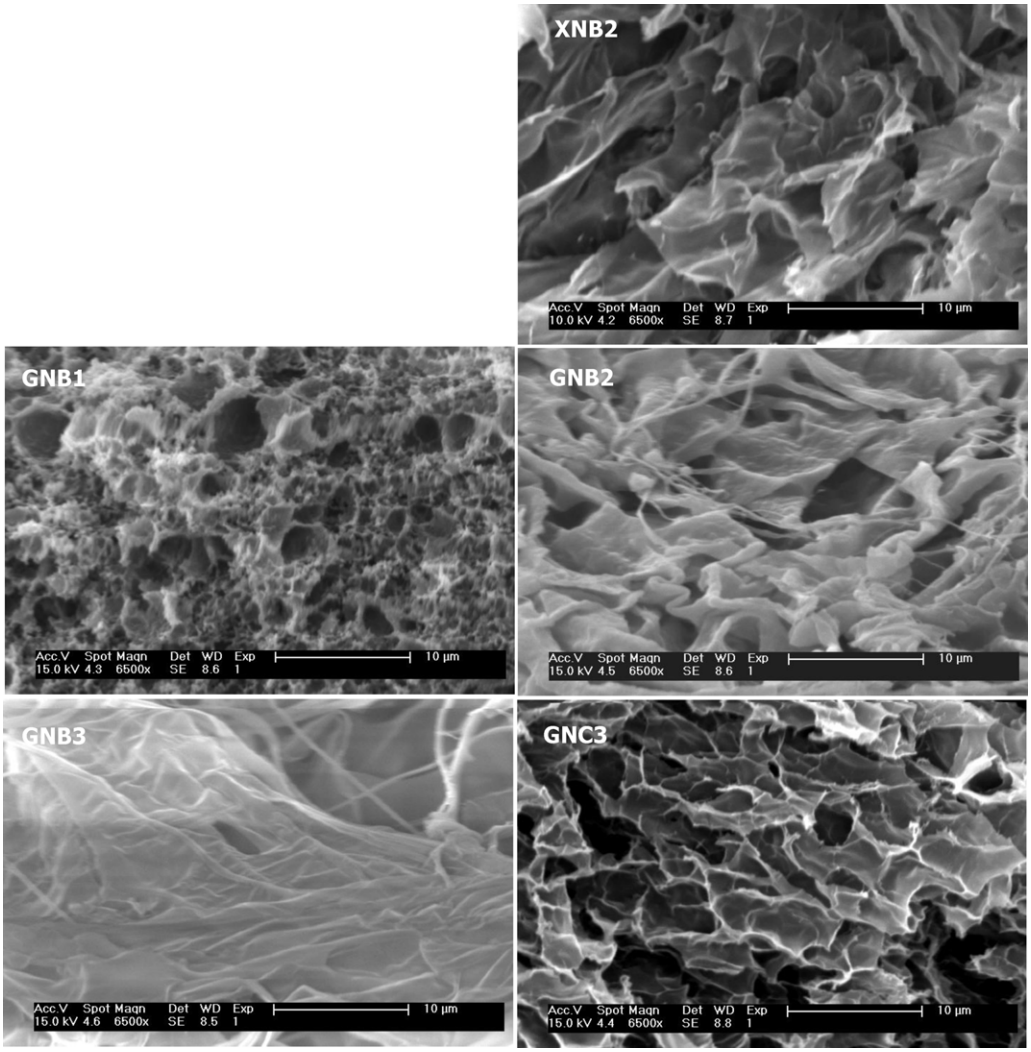


Fig. 3. Scanning electronic microscopy micrographs for a series of freeze-dried hydrogels based on MA-P synthesized with BIS or A-CD as the crosslinking agent.

released when the organism manifests a febrile state, due to an infection.

LCST determination of elaborated thermosensitive systems was usually performed by differential scanning calorimetry (Schild, 1992), cloud-point measurements (Freitag & Garret-Flaudy, 2002) or more recently ^{1}H High Resolution Magic Angle Spinning (HRMAS) NMR spectroscopy (Rice, 2006). The DSC technique was used in the present study to determine and compare the LCST values of the polysaccharide–PNIPAm hydrogels. The obtained data are given in Table 3.

LCST increases with polysaccharide content, the use of cyclodextrin acrylate as the crosslinking agent leading to its more pronounced increase. This is related to the high number of hydroxyl groups of cyclodextrin able to form more hydrogen bonds than hydrogels without this additive. Gellan based hydrogels exhibit larger LCST than Xanthan based hydrogels with the same composition. This may be explained by the lowest stiffness of the Gellan macromolecular chain and the smallest number of hydrogen bonds able to be formed per repeating unit (with 10 hydroxyl groups and 4 glycosydic oxygen atoms for Gellan compared with 11 hydroxyl groups and 5 glycosydic oxygen atoms for Xanthan).

Due to the presence of weak acid carboxylic groups on the polysaccharide chains, the hydrogels are also sensitive to pH and ionic strength. The pH values have a minor effect on the LCST values in the pH range from 3 to 9.6 for samples of given composition (Supporting information). By contrast high NaCl concentrations lead to a noticeable decrease of the LCST values (Supporting information). This is related with the water structuring or destructuring effect of salts, a well-known phenomenon (Pastoor & Rice, 2012; Yang, Zeng, Tong, Liu, & Wu, 2001).

Table 3
LCST values in water of hydrogels based on polysaccharide maleate (MA-P)–PNIPAm determined by DSC.

Samples codes	MA-P/NIPAm ratio (%, wt/wt)	LCST (°C)
NB0	0–100	34.2
GNB1	10–90	33.7
GNB2	25–75	34.1
GNB3	50–50	35.4
GNC1	10–90	33.9
GNC2	25–75	35.7
XNB1	10–90	30.2
XNB2	25–75	32.3
XNB3	50–50	34.8
XNC1	10–90	31.5
XNC2	25–75	35.1
XN1	25–75	31.8

The concentration of polysaccharide maleate is 2.5% (wt/v) for all the hydrogels. The concentration of the crosslinking agent (BIS or A-CD) is 3% (wt/wt) with respect to comonomers mixture. XN1 was prepared without any crosslinking agent.

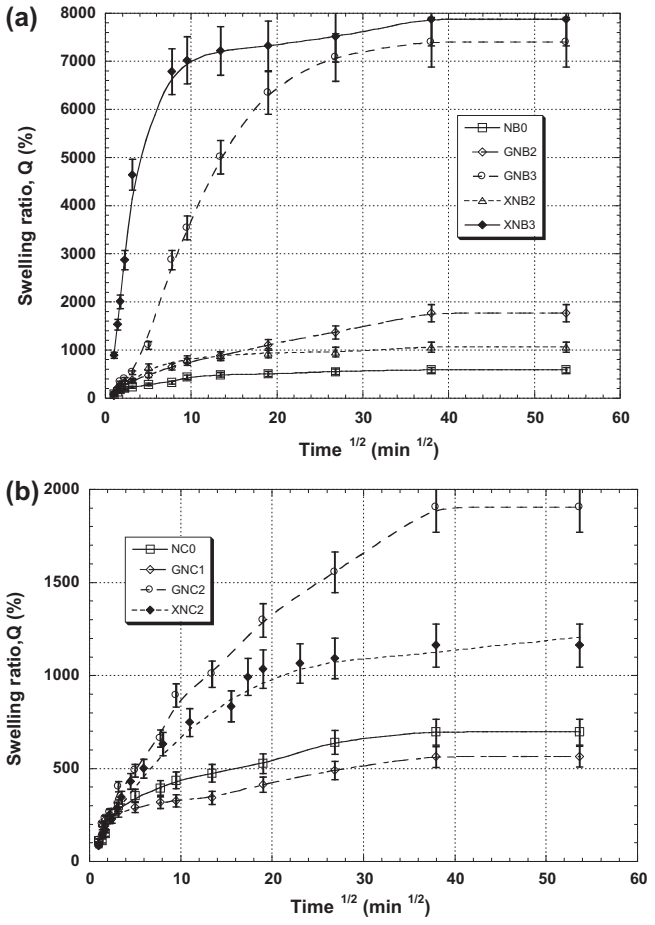


Fig. 4. Swelling kinetics of MA-P/PNIPAm based hydrogel at 25°C in distilled water: (a) with BIS as the croslinking agent, (b) with A-CD as the crosslinking agent.

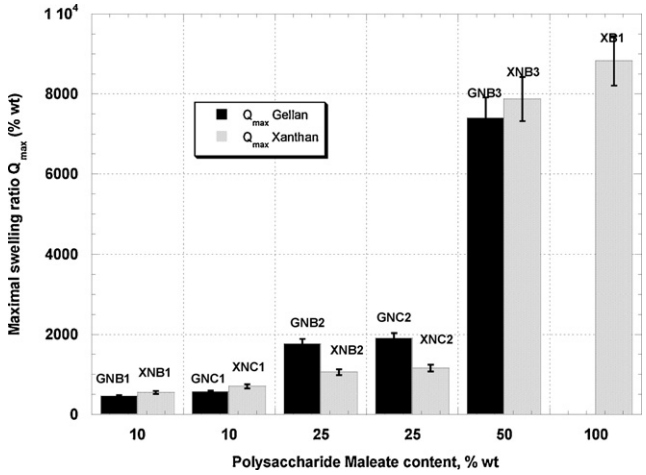


Fig. 5. Effect of the polysaccharide maleate content and of the nature of the crosslinking agent on the maximal swelling degree at room temperature in distilled water.

3.5. Swelling properties of hydrogels

The aimed application of such hydrogels being, at first, the inclusion and the release of biologically active matter the swelling properties are of major importance. The swelling behaviour of hydrogels depends on different parameters such as the crosslinking degree, the presence (or absence) of specific interactions between comonomers and the medium in which they are inserted. These are generally taken into account in the classical theories as those of Flory–Rehner, or in a statistical mechanical analysis of gels (Panyukov & Rabin, 1996a, 1996b). The classical theory was based both on the phamton network and affine network models and the ideal Donnan theory (Caykara & Akçakaya, 2006). The basic assumptions of additivity of the different osmotic contributions have been scrutinized. It is now accepted that the osmotic pressure of crosslinked polymers can differ from that of the uncrosslinked ones of the same chemical nature. However, and especially for polyelectrolyte systems, the swelling of hydrogels is furthermore influenced by the charge density. However, due to the complex two-phase structure of the present hydrogels these theoretical aspects could not directly be taken into account in the discussion of the results. The following will therefore be mostly a comparative study of the hydrogel swelling characteristics in view of their practical application possibilities. Fig. 4(a, b) displays typical examples of the swelling kinetics of the MA-P/PNIPAm hydrogels as a function of the square root of time for BIS and cyclodextrin acrylate as crosslinking agents respectively.

The kinetic swelling curves present the typical shape of limited swelling networks. The observed curves are very similar to those obtained by Caykara and Akçakaya (2006) which demonstrate a Fickian behaviour of water diffusion. Due to the initial linear dependence of the swelling ratio with the square root of time, it may be concluded that it is initially controlled by the diffusion rate of water molecules. Apart from the hydrogels with the larger polysaccharide content, the nature of the polysaccharide has only a slight influence on the kinetics. Moreover, by comparing the crosslinking agents it becomes possible to discriminate the role of their respective functionality and their consequence on the crosslinking density (at equivalent molar concentration). Cyclodextrine acrylate has an average functionality of 3; the swelling kinetics of these hydrogels is lower compared with that obtained with BIS, which functionality is equal to 2. From the maximal values obtained these hydrogels may be classified as “superabsorbants”, these values being much larger than usual hydrogels. At low polysaccharide content (10%) the kinetics is slightly similar to that of crosslinked PNIPAm prepared in absence of polysaccharide (not shown). For a content of 25% the Gellan based hydrogels (GNB2 and GNC2) have an enhanced swelling rate compared to those prepared with Xanthan (XNB2 and XNC2 respectively) whereas at higher polysaccharide contents it seems that Xanthan based hydrogels present a higher initial swelling rate (XNB3 compared to GNB3). These observations will be found again on the maximal swelling ratio.

The effect of modified polysaccharide macromonomer ratio and of the crosslinking on maximum swelling degree of hydrogels is summarized in Fig. 5. The maximal swelling increases with the polysaccharide content in relation with the higher hydrophilicity of polysaccharide compared with PNIPAm. As already observed with Xanthan a characteristic transition occurs between 25 and 50% of MA-P [24]. It is typical for nano- or microphase separated interpenetrated network systems as shown by Manson and Sperling (Manson & Sperling, 1976, chap. 8). At low MA-X or MA-G content, a co-continuous network structure exists with PNIPAM as predominant phase. In contrast at high contents of crosslinked MA-X or MA-G, the reverse situation occurs with the polysaccharide as the major network phase.

Further observations can be made concerning the swelling behaviour:

- hydrogels crosslinked with BIS and A-CD have Q_{max} values decreasing with an increase of the crosslinking agent concentration with respect to the comonomer mixture, due to the increase of the crosslinking density,

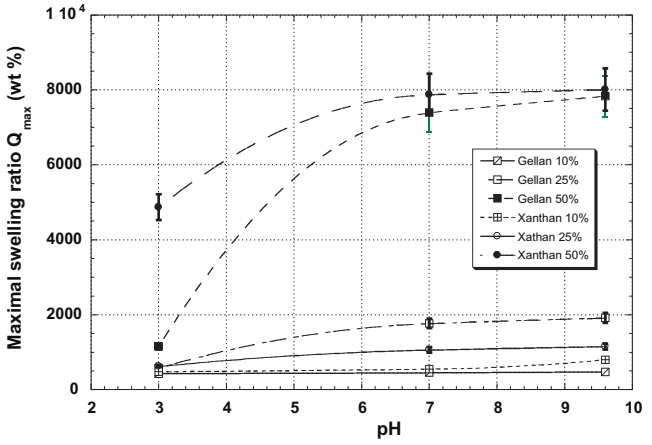


Fig. 6. Maximal swelling degree of polysaccharide maleate/PNIPAm hydrogels crosslinked with BIS as a function of pH and polysaccharide maleate content at room temperature.

- for a weight ratio polysaccharide maleate/NIPAm equal to 10/90, the Gellan based hydrogels (samples GNB1 and GNC1) show lower values of Q_{max} as compared to Xanthan based hydrogels (samples XNB1 and XNC1) due to a higher crosslinking density as a consequence of the higher functionality of the Gellan ester,
- for weight ratios higher than 25/75, two effects are counterbalanced: the rigid Xanthan chains lead to an increased stiffness of the network and a decrease of swelling on one part, the higher functionality of the Gellan ester leads to a decrease of the swelling on the other part. As a consequence, according to the composition, one or the other effect is predominant.

The nature of the crosslinking agent has a slight influence on the swelling efficiency of the hydrogels (Fig. 5). Hydrogels crosslinked with cyclodextrin acrylate (A-CD) swell more than those prepared with BIS (PNC1 and PNC2 samples compared with PNB1 and PNB2 ones respectively). This is explained by the difference in molar concentrations for BIS and A-CD and by the highest hydrophilicity of cyclodextrin derivative, related to the presence of non-esterified hydroxyl groups in the former.

Due to the presence of a weak acid group on the macromolecular backbone of the polysaccharides they act as polyelectrolytes. As a consequence, the swelling of hydrogels is sensitive both to pH and electrolytes. The influence of pH is demonstrated in Fig. 6. The curves are similar to those obtained by Caykara and Akçakaya (2006) with ionic hydrogels based on N,N-dimethylacrylamide, acrylamide and itaconic acid. However, due to the high complexity of our hydrogels it was not possible to fit our curves with their model and, as a consequence, to determine pertinent parameter values to be compared with their ones. Increasing pH leads to ionization of the carboxylic groups (those from the polysaccharide chain as well as those obtained by the functionalization reaction of maleic anhydride) and thus to an increase in the charge density of the components of the hydrogel. As a consequence the electrostatic repulsion between the ionic charges leads to expansion of the hydrogels that implies an increase of swelling. At high pH (higher than the pKa of the carboxylic groups on the macromolecular backbone), they are anionic and the swelling does not differ significantly between the Xanthan- and the Gellan-based hydrogels. On the contrary at low pH (pH 3) the carboxylic groups are under non ionic form and the Gellan-based hydrogels present a lower swelling power than Xanthan-based hydrogels, which is especially observed for high polysaccharide contents. This is due to the lowest Gellan chain stiffness leading to more entangled systems and hence more hydrogen-bonding. As a consequence the swelling will be reduced.

Table 4
Swelling characteristics of Xanthan and Gellan-based hydrogels in aqueous solutions at different electrolyte concentrations.

Sample	[NaCl] = 0 mol L^{-1}	[NaCl] = 0.005 mol L^{-1}	[NaCl] = 0.05 mol L^{-1}
XNB2	1060	590	330
XNB3	7880	5130	3120
GNB2	1770	1320	1180
GNB3	7400	5640	3430
GNC2	1165	1025	935
XNC2	1905	1410	1140

As well as pH, the electrolyte content is one of the key parameters in the swelling of hydrogels, when polyelectrolytes are present within the crosslinked system. The sensitivity to electrolyte solutions differs either by their concentration (Table 4) or by the nature of ions. The composition of the lachrymal liquid is very complex and it consists of water, electrolytes, proteins, vitamins, etc. Considering the electrolytes, sodium ions are predominant and its concentration is in the 0.05–0.15 mol L^{-1} range. The presence of monovalent ions such as Na^+ leads to a decrease of Q_{max} as compared to water. This well-known effect, a typical polyelectrolyte one, is due to the decrease in electrostatic interactions by electrostatic screening. The larger the ionic concentration, the more observed is this effect. The lowest swelling is observed for the highest NaCl concentration studied. As we had already observed in water and pH-dependent solutions (Hamcerencu et al., 2009) the effect of a structural change in the hydrogel is observed between 25 and 50 wt% of MA-P. For higher content, the polysaccharide becomes the predominant phase of the hydrogel. As a consequence, its higher hydrophily, sensitivity to pH and ions are the most important parameters.

In aqueous salt medium the Xanthan based hydrogels show a lower swelling than Gellan based systems. This may be explained by the fact that room temperature, at which swelling is determined, is lower than the conformational change temperature considering the polymer and salt concentrations (Milas & Rinaudo, 1986). As a consequence the Xanthan chain is under ordered conformation and much more rigid. Moreover, its charge density being larger compared with Gellan, its electrostatic screening effect will decrease swelling in higher proportions. Finally, by comparing XNC2 and XNB2 samples it is demonstrated that the A-CD crosslinked hydrogels are less sensitive to electrolytes compared with those crosslinked with BIS.

4. Conclusion

Hydrogels based on polysaccharide maleate/N-isopropylacrylamide, in a broad compositional range, were elaborated by a grafting-crosslinking reaction using either N,N′-methylenebisacrylamide (BIS) or cyclodextrin acrylate (A-CD) as the crosslinking agent. Well-defined interpenetrated polymer networks were obtained and a reaction scheme was proposed. The temperature, electrolyte and pH-responsive behaviour of these hydrogels were demonstrated by differential scanning calorimetry and by equilibrium swelling experiments. The role of the different components on the properties, such as LCST, morphology and swelling ability, was investigated. It turned out that A-CD, as compared to BIS crosslinking, leads to hydrogels with increased LCST. It appeared furthermore that the predominant co-continuous phase, PNIPAm or polysaccharide, of the polymer network, determines the swelling characteristics of the hydrogel as a function of pH and electrolytes. The role of the polysaccharide depends upon the polysaccharide content and A-CD crosslinked hydrogels show a larger swelling than BIS-crosslinked ones at similar composition. It could further be demonstrated that the stiffness of the polysaccharide backbone and its charge density,

as well as its ability to form hydrogen bonds, influence LCST and swelling behaviours of the prepared hydrogels. Considering inclusion and delivery applications within human body, the nature and the salt concentration of the swelling medium are of major importance. In this context A-CD crosslinked hydrogels are less sensitive to electrolytes than those obtained with BIS.

Further studies of this type of hydrogels concerning their biocompatibility, *in vivo* biodegradability, ability to include and release water- and liposoluble drugs, as well as their antimicrobial activity, will be considered in a future paper.

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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.carbpol.2012.03.026.References

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