Polym. Bull. (2009) 62:525–538
DOI 10.1007/s00289-008-0033-8

ORIGINAL PAPER

Trisodium trimetaphosphate crosslinked xanthan networks: synthesis, swelling, loading and releasing behaviour

Anca Bejenariu · Marcel Popa · Virginie Dulong · Luc Picton · Didier Le Cerf

Received: 19 May 2008 / Revised: 15 December 2008 / Accepted: 16 December 2008 /
Published online: 8 January 2009

Abstract Xanthan (Xan) is a biocompatible and biodegradable polysaccharide with a promising potential as substrate for controlled drug delivery applications. Xan based hydrogels were synthesized in alkaline medium using trisodium trimetaphosphate (STMP) as crosslinking agent. Hydrogels with various crosslinking agent/polymer ratios were synthesized and subsequently characterized by the means of elemental analysis and dynamic swelling degree, model compound loading and releasing behaviour. Two physical parameters (crosslinking density and phosphate charge) are manifesting antagonistic actions by stiffening or disrupting the three-dimensional macromolecular ensemble. The highest swelling degree was obtained using an intermediate STMP:Xan ratio in which case the opposing effects of the two forces are well balanced. The synthesized networks are pH sensitive. In acid and alkaline media the swelling degrees are lower by comparison to neutral pH. The entrapping and releasing behaviour of the newly synthesized xanthan networks were studied using methylene blue as a cationic model molecule. The releasing kinetics present a first-order model.

Keywords Polysaccharide · Xanthan Hydrogel · Trisodium trimetaphosphate · pH dependance

A. Bejenariu · M. Popa
Faculty of Chemical Engineering and Environmental Protection,
Department of Natural and Synthetic Polymers,
Technical University "Gh. Asachi" Iasi,
Bd. D. Mangeron, Nr. 71A, 700050 Iasi, Romania

A. Bejenariu · V. Dulong · L. Picton · D. Le Cerf (✉)
FRE 3101 & FR 3038 CNRS Polymères Biopolymères Surfaces,
University of Rouen, 76821 Mont Saint Aignan, France
e-mail: didier.lecerf@univ-rouen.fr

Introduction

Synthetic polymers based gels are excellent water absorbants. Quite often their toxicity and carcinogenicity, determined by residual monomers, low molecular weight fractions resulting from oxidative metabolic processes, traces of organic solvents used during synthesis, separation and purification, are limiting their usage in applications were ecological issues or biocompatibility are of interest.

By comparison, polysaccharides are biocompatible and biodegradable natural occurring macromolecules that have been successfully utilized in various industrial and medical domains: oil, food, cosmetic, consumer products, pharmaceutical, etc. They have specific physico-chemical properties that determined their application as rheological controllers [1], surfaces modifiers [2] or substrates for controlled drug delivery systems [3]. The presence of a plurality of functional groups such as OH, COOH, or NH_2 explains their usual water solubility and by crosslinking these can be used for obtaining stable macromolecular networks.

Xanthan (Xan) is a bacterial exopolysaccharide produced by *Xanthomonas campestris* [4] with important capacities to thicken, emulsify, stabilize, flocculate, swell and suspend the aqueous solutions in order to form gels, films and membranes [5]. Its main chain consists in units of D-glucose linked in β-1,4 positions and every alternate glucose unit has a side chain consisting of β-D-mannose-(1,4)-β-D-glucuronic acid-(1,2)-α-D-mannose. The internal mannose unit may be acetylated in C-6 position and the terminal mannose may carry pyruvate residues linked in 4- and 6-positions [6, 7].

From the secondary structure point of view, depending on statistical parameters such as temperature, ionic strength and pH, xanthan may have one of the conformations: an ordered, rigid helix or a disordered, flexible coil. The ordered conformation can be either native or renatured. The native xanthan is in the ordered conformation as a right-hand helix with a fivefold symmetry, a pitch of 4.7 nm and a diameter of 1.9 nm [8]. The helix is stabilized by inter and intramolecular hydrogen bonds [9]. In aqueous solutions, xanthan presents a conformational transition from the ordered helical shape at physiologically relevant temperatures and salt concentrations to a disordered chain conformation at elevated temperatures and low ionic strength. The transition temperature may determine a complete or a partial separation of the double-strand form. The reverse process in which the statistical coil is transformed back into the ordered helix is named renaturation. It occurs when the temperature decreases below the transition temperature ($T \ll T_m$) and the solution has a high ionic strength**.** The renaturation phenomenon is a reversible one, while the denaturation of native xanthan is irreversible [10, 11].

While in solution, xanthan is able to form both physical and chemical gels. The physical gels based are not practical to be used as drugs carriers because they can easily be solubilised in aqueous solution [12]. Chemical networks based on xanthan concentrated solutions should provide an economically feasible solution for the pharmaceutical domain. In order to obtain a macromolecular substrate that maintains its physical dimensions xanthan needs to be chemically crosslinked.

In order to synthesize xanthan based networks in alkaline media, different crosslinking agents were used [13, 14] but their action was found to be toxic and carcinogenic [15].

Trisodium trimetaphosphate (STMP) is a non-toxic cyclic triphosphate [16], already reported as an effective crosslinker for starches [17, 18], guar gum [19], hyaluronic acid [20], pullulan [21] or pullulan derivates [22] in order to synthesize gels for pharmaceutical purposes. After long debates [20], recently the STMP reaction mechanism was clarified through an NMR study [23, 24].

In the view of the above, the present study proposes an organic solvent free xanthan network synthesis performed in strong alkaline conditions using cyclic trisodium trimetaphosphate (STMP) as crosslinking agent. It has been demonstrated that the equilibrium helix/coil can be modified by external parameters (pH, temperature and ionic strength) either towards a preponderantly ordered conformation (acid pH, low temperature) or a dominantly disordered one (alkaline pH, high temperature, low ionic strength) [25]. The swelling properties of the synthesized hydrogels were monitored in solutions with various pH values and different behaviours have been observed. In addition to pH sensitiveness, the drug loading and releasing behaviour were assessed in body mimicking fluids using a small molecule drug as a model for biologically active substances. In this manner, an organic solvent free synthesis was conducted in order to obtain potentially biocompatible hydrogels.

Experimental

Materials

Xanthan (Rhodicare) was provided by Rhodia (France). Its molecular weight (M_w) was determined by size exclusion chromatography with on line multi angle laser light scattering and it was found to be 2×10^6 g mol^{-1}. The substitution degrees with acetate and pyruvate determined by ^{1}H-NMR study were 1 and 0.6, respectively [26]. Lithium nitrate ($LiNO_3$) was provided by Kelkogel. STMP and methylene blue (MB) were purchased from Sigma-Aldrich, while sodium hydroxide (NaOH) was provided by VWR. All compounds were used without any further purification and for all the experiments Milli-Q water (mQ) was used as solvent.

Rheological measurements

In order to determine the influence of the temperature and pH over the helix-coil transition, rheological measurements were performed on a concentrated xanthan solution, using a controlled stress rheometer (AR2000 TA Instrument) with cone plate geometry and a solvent trap. The xanthan solution was obtained by dissolving a determined quantity of xanthan (25 g) in 1 litre $LiNO_3$ solution, 10^{-3} mol L^{-1}. $LiNO_3$ was chosen to be the solvent media because of the convenient to proceed other characterisation methods (i.e., Size Exclusion Chromatography). The solutions pH was adjusted at 13 adding NaOH, 5 mol L^{-1},

(alkaline synthesis) and 3 (HCl, 1 mol L^{-1}). Oscillatory dynamic experiments were subsequently performed.

Hydrogels synthesis

Xanthan networks were obtained by a crosslinking reaction with STMP performed in alkaline conditions. Solutions with two different xanthan concentrations (10 or 25 g L^{-1}) in $LiNO_3$ (10^{-3} mol L^{-1}) were obtained under magnetical stirring. The solutions pH was fixed at 13 with NaOH, 5 mol L^{-1}. The solutions were heated up to 90 °C and after 1 h the STMP was added. The STMP:Xan molar ratios were 1:1, 5:1, 10:1, 15:1 and 20:1. From this point onward, throughout the paper, the molar ratios will be mentioned as 1, 5, 10, 15 and 20, respectively. The obtained solutions were left under vigorous magnetical stirring for 24 h. The obtained gel was purified by successive dialysis in mQ. The purified product was precipitated in methanol and dried in the oven at 40 °C. The dried gels were kept in a desiccator until used.

The synthesis yield was calculated according to Eq. 1:

$$\rho(\%) = \left(\frac{m_p}{m_i}\right) \times 100 \tag{1}$$

where ρ (%) is the yield of the reaction, m_i is the amount of xanthan introduced initially into the reaction and m_p is the final amount of dried gel obtained after precipitation.

Swelling properties of the synthesized gels

In order to analyze the swelling behaviour, a weighed quantity of dried gel was added into a large volume of mQ water. At determined time intervals the gel was separated, gently wiped to remove the excess adsorbed solvent and weighed. Using Eq. 2, the swelling degree (Q) and consequently the swelling kinetics were assessed.

$$Q = \frac{m - m_0}{m_0} \tag{2}$$

where m_0 is the weight of the dried gel and m is the weight of the swollen gel. Q was considered to be at equilibrium (Q_e) when identical values of Q were recorded during consecutive measurements (Q constant). All the gels were synthesized using a 25 g L^{-1} Xan solution. During experiments, the macromolecular networks used had a 5, 10 or 15 STMP:Xan ratio and the temperature was kept constant at 25 °C. The influence of parameters such as solvent pH and STMP:Xan ratio was hence investigated.

Drug loading and releasing

A weighed amount of dried gel was immersed in 30 ml of methylene blue (MB, 2×10^{-5} mol L^{-1}) and left for 24 h to reach equilibrium. The gel was subsequently removed and dried. The drug release kinetics was assessed in two body-mimicking fluids: an acid solution with a pH 1.5 (HCl, 1 mol L^{-1}), similar to

that of the stomach and a saline solution with a salt concentration similar to the physiological serum (NaCl, 0.1 mol L^{-1}). A weighed quantity of gel loaded with MB was immersed in 30 mL of releasing aqueous medium. For this, at regular time intervals, 3 mL aqueous medium were extracted (and replaced after measurement) from the releasing flask and the absorbance at 664 nm was measured using an UVIKON 860 (Kontron Instruments) spectrophotometer. The variation in time of the MB concentration obtained with calibration curve was monitored.

For the entrapping and releasing kinetics, the MB percentage variation was calculated as follows:

$$\mathrm{MB}^r = \frac{\mathrm{MB}_g^r}{\mathrm{MB}_s^r} \times 100 \tag{3}$$

$$\mathrm{MB}^e = \frac{\mathrm{MB}_g^e}{\mathrm{MB}_s^e} \times 100 \tag{4}$$

where MB^e, and MB^r (%) are the percentages of drug entrapped and released, respectively, MB_g^e and MB_g^r (g) represent the weight of MB from the gel during entrapping and releasing and MB_s^e and MB_s^r (g) are the weights of MB in solution during entrapping and releasing.

Results and discussion

Rheological behaviour of concentrated xanthan solutions

In order to determine the optimal parameters for a consequent high yield crosslinking reaction, frequency and temperature evolution measurements were performed using a concentrated Xan solution (25 g L^{-1}), different pH (3 and 13) and temperature conditions (25 and 90 °C; Fig. 1). Storage (G') and loss (G'') moduli variations with frequency and temperature were recorded.

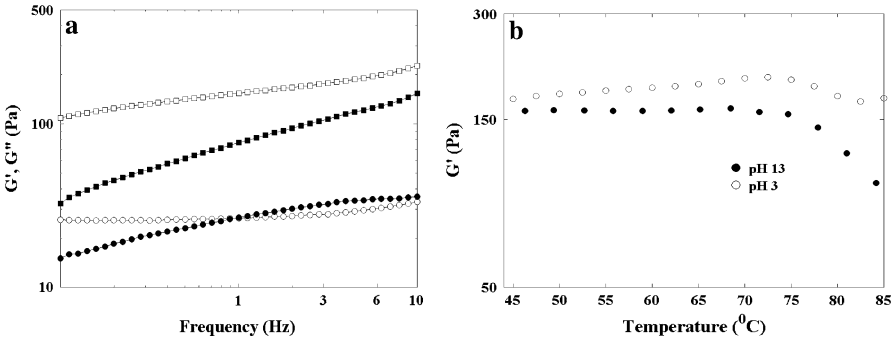


Fig. 1 **a** Frequency variation of storage (*squares*) and loss (*circles*) moduli, 25 g L^{-1} Xan, pH 13 and different temperatures: 25 °C (*white marks*) and 90 °C (*black marks*), logarithmic scale; **b** Temperature variation of storage modulus at pH 3 and 13, 25 g L^{-1} Xan, logarithmic scale

In the case of 90 °C, within the analyzed frequency range, G' registered values that were between 3.42 (0.1 Hz) and 1.5 (9 Hz) times lower than the ones recorded at 25 °C (Fig. 1a). This indicates a temperature driven ordered-disordered conformational transition. As a result of this process, at equilibrium, the coil domains are coexisting with the helical ones within the concentrated Xan solution. In the case of pH 3, the temperature ramp experiments demonstrated that G' has a quasi linear variation with temperature. At pH 13 the presence of an inflexion point that locates a severe drop in the G' amplitude was determined. This suggests that the helix-coil conformational transition temperature is positioned at approximately 75 °C (Fig. 1b).

The rheological results are indicating that, in the case of concentrated solutions, xanthan is predominantly in its coiled, flexible and disordered conformation when the pH of solution is 13 and the temperature above 75 °C.

Hydrogels synthesis

Considering the application of the gels as macromolecular substrates for controlled drug delivery systems, the Xan networks were synthesized via a solvent free approach avoiding hence the use of any potentially toxic organic solvents during synthesis, separation and purification. The non-toxic STMP was used as cross-linking agent and the reactions were performed in a low salt concentration aqueous solvent ($LiNO_3$ in Milli-Q). The entire synthesis process was designed to obtain gels in which the Xan is predominantly in its flexible, coil conformation. To achieve this purpose, the following experimental conditions were selected based on rheological analysis results: high temperature, 90 °C, low ionic strength, 10^{-3} mol L^{-1} $LiNO_3$ and pH 13.

The Xan-STMP crosslinking reaction starts with the transformation of xanthan hydroxyl groups into alcoholates as a result of the NaOH presence (Fig. 2a). The alkaline conditions are determining the degradation of STMP by opening of the triphosphate cycle. The consequent formation of the Xan grafted sodium tripolyphosphate ($STPP_g$) represents an intermediate stage within the crosslinking reaction (Fig. 2b). At the same reaction step, non-grafted STPP, a secondary by-product, is also obtained to a certain extent. At the next reaction step, $STPP_g$ reacts with Xan alcoholate groups forming a mixture of mono (P_g) and diester phosphates (P_c) (Fig. 2c). The inorganic pyrophosphate (PP_i) is also obtained as secondary product and is subsequently removed by gel dialysis.

In order to maximize the yield of xanthan gel synthesis, the polysaccharide concentration and STMP:Xan molar ratios were varied (Table 1). According to our preliminary synthesis and also to previous works [21, 23], the reaction time was fixed to 24 h since lower synthesis times lead to poor crosslinking yields.

A Xan concentrations of 10 g L^{-1} and a STMP:Xan molar ratios of 1 did not determine insoluble gel formation. Increasing the STMP:Xan ratios above to 5, 10 and 20 gradually increased the reaction yields to acceptable values. The STMP cycle opening is sensitive to changes in pH and in order to prevent the decay of the crosslinking reaction rate, the alkalinity of the medium was regularly adjusted to 13 using a 5 mol L^{-1} NaOH solution [23].

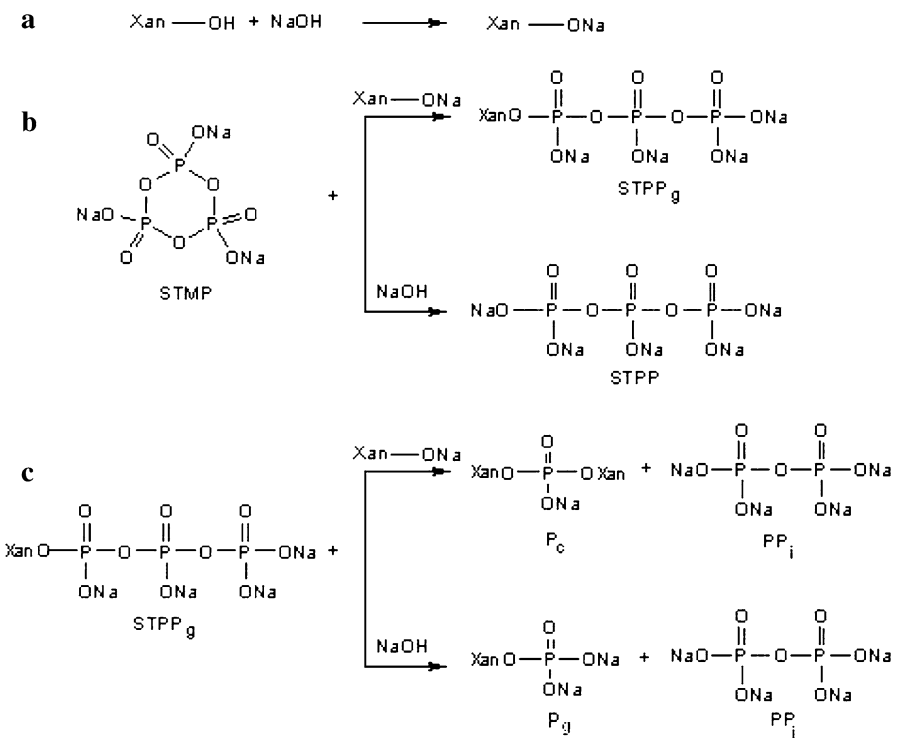


Fig. 2 Xan-STMP crosslinking reaction: **a** Alcoholate formation, **b** opening of the STMP cycle, **c** crosslinking reaction and the degradation of $STPP_g$ [21]. *STMP* trisodium trimetaphosphate, *STPP* sodium tripolyphosphate, $STPP_g$ grafted sodium tripolyphosphate, P_c phosphate diester, P_g phosphate monoester, PP_i inorganic pyrophosphate

Table 1 Crosslinking reaction yield for the synthesized Xan gels Legend: nXpS, *n* is Xan concentration (g/L) and *p* represents the value of STMP:Xan molar ratio; ρ (%), synthesis yield

Sample	10X1S	10X5S	10X10S	10X20S	25X5S	25X10S	25X15S
ρ (%)	–	25	30	35	35	30	47

Considering a supplementary synthesized gels quantitative increase, a higher Xan solution concentration, 25 g L^{-1}, was experimented. In this case the highest yield (47%) was obtained for a STMP:Xan ratio of 15. For all the consecutive analyses, only the gels based on a 25 g L^{-1} Xan solution were monitored since they were the most productively attractive.

Because the crosslinking is realized through phosphate esters, the phosphorus elemental analysis is a valuable estimator of the synthesis efficacy. The results of carbon and phosphorus elemental analysis are presented in Table 2.

For all the analyzed gels, the elemental analysis revealed the presence of phosphorus, its concentration registering a gradual increase with the increase of the STMP:Xan molar ratio. The phosphorus presence determines an increase of the

Table 2 Carbon and phosphorus elemental analysis results

Element	Xan	25X5S	25X10S	25X15S
C (%)	34.3	34.1	33.6	33.3
P (%)	–	0.7	0.8	1

anionic charges in the gels. This affects their physical behaviour in a manner that will be detailed bellow.

Swelling behaviour of xanthan gels

Hydrogels are three-dimensional insoluble networks, chemically or physically crosslinked, presenting the capacity to imbibe a large amount of solvent that it is retained when no pressure is applied onto them. Therefore, the swelling measurements are a good method for the physical characterisation of the crosslinked polysaccharide networks. The swelling degree is a process influenced both by the crosslinking density and the electrostatic repulsions exerted between molecular chains. In order to evaluate the swelling kinetics for the selected gels, the experiments were firstly performed in a pH 7 aqueous environment. The swelling kinetics is presented in Fig. 3a.

It can be noticed that the swelling process reaches equilibrium after approximately 80 min and it remains constant for the rest of the time interval. The magnitude of the swelling process is heavily influenced by two antagonistic phenomena: the crosslinking which reduces the swelling by stiffening the macromolecular matrix and the phosphate anionic charges which are generating electrostatic repulsions between the neighbouring chains of the polysaccharide, thus having a positive effect over swelling. In our case, the highest swelling degree, approximately 120 g g^{-1}, was obtained in the case of an intermediate STMP:Xan ratio (10). For a lower ratio (5), the phosphorus concentration in the networks is the lowest (0.7%) and its influence over the swelling degree is minor leading to the lowest *Q* at equilibrium, approximately 35 g g^{-1}. In the case of a higher STMP:Xan

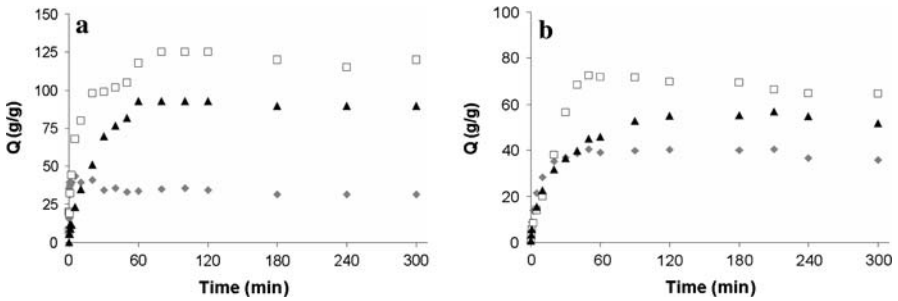


Fig. 3 Swelling kinetics at pH 7 (**a**) and at pH 3 (**b**): 25X5S (*rhombus*), 25X10S (*square*), 25X15S (*triangle*)

ratio (15), the electrostatic repulsion is overshadowed by a high crosslinking density that reduces sharply the swelling degree, 85 g g^{-1}.

A similar swelling behaviour was registered at pH 3 (Fig. 3b). In terms of maximum swelling hierarchy, the order is not modified: 25X5S < 25X15S < 25X10S. In the case of 10 and 15 STMP:Xan ratios, Q is though 1.7 and 1.5 time smaller by comparison to pH 7. This can be explained by taking into account that the crosslinked STMP/Xan hydrogels are having anionic phosphate groups. At high pH values the phosphate groups become ionized and the electrostatic repulsive forces are causing an increase in swelling. At acid pH such as 3, the protons will screen the majority of the phosphate anions diminishing the repulsive interactions with the decrease of Q. In addition to this, a coil-helix conformational transition may also occur at pH 3 and have an additive effect in decreasing swelling.

In alkaline conditions (pH 13), Na^+ cationic counterions (concentration 0.1 mol L^{-1})have a screening effect that shields the anionic electrostatic repulsions determining the collapse of the gel, which in turn causes a dramatic drop in swelling at equilibrium. This phenomenon was observed for all the investigated materials (Fig. 4).

MB: entrapment and releasing behaviour

Methylene blue is a cationic molecule with a high affinity for negatively charged polymers (Fig. 5). Due to this it was chosen as a model molecule for evaluating xanthan gels capacity to entrap and release biologically active substances.

To maximize the loading capacity, each of the analyzed weighed dried gels was immersed in a MB solution of known concentration (2×10^{-5} mol L^{-1}) and the variation of the characteristic absorbance peak (664 nm) was measured. The final value of the absorption was registered after 24 h. The MB absorption kinetics follow a pattern similar to the swelling since the process is driven by the passive diffusion of the MB into the hydrogels and influenced by the balance crosslinking density/

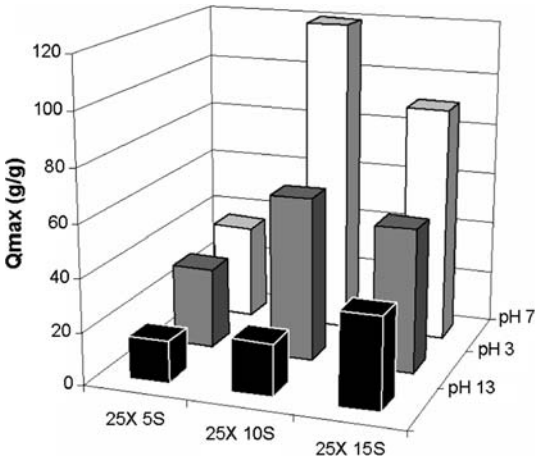
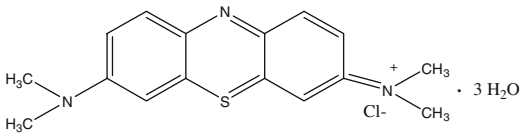


Fig. 4 Variation of the equilibrium swelling degree in function of STMP:Xan ratio and solution pH

Fig. 5 Chemical structure of methylene blue



anionic charges. The percentage time variation of MB into the hydrogels normalized with the initial MB amount in the solution represents the effective measure of the substrates entrapping properties (Fig. 6). The process reaches equilibrium after approximately 80 min and at this stage significant amounts of MB are incorporated in the hydrogels: 80, 87.5 and 85% corresponding to the STMP:Xan molar ratios 5, 10 and 15, respectively.

Based on the physical or chemical characteristics of polymer/polysaccharide, drug release mechanism from a polymer matrix can be categorized in three main processes (systems) [27]: (1) diffusion-controlled system, (2) swelling-controlled system, (3) erosion-controlled system. This categorization allows mathematical models to be developed and various kinetic models were used to describe the release kinetic. The zero order rate Eq. 5 describes the systems where the drug release rate is independent of its concentration [28]. The first order Eq. 6 describes the release from system where release rate is concentration dependent [29]. Higuchi [30] described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Eq. 7. The Hixson–Crowell cube root law Eq. 8 describes the release from systems where there is a change in surface area and diameter of particles or tablets [31]

$$C = k_o t, \tag{5}$$

where, C is the concentration of drug released in time t, k_0 is zero-order rate constant expressed in units of concentration/time and t is the time;

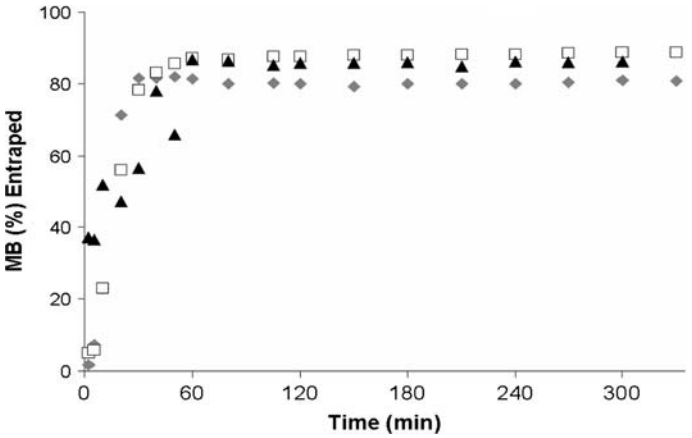


Fig. 6 MB entrapping behaviour: time variation of the MB percentage in the hydrogels with respect to the initial quantity in the solution. 25X5S (*rhombus*), 25X10S (*square*), 25X15S (*triangle*)

$$\log C = \log C_0 - kt/2.303, \tag{6}$$

where, C_0 is the initial concentration of drug and k is first order constant;

$$Q_t = Kt^{1/2}, \tag{7}$$

where, Q_t is the amount of drug released in time t, K is the constant reflecting the design variables of the system;

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC}t, \tag{8}$$

where, Q_0 is the initial amount of the drug in tablet and K_{HC} is the rate constant for Hixson–Crowell rate equation.

The releasing kinetics was investigated in physiologically relevant human body mimicking fluids: a solution with the pH 1.5 similar to the gastric medium and a NaCl 0.1 mol L^{-1} solution similar to physiological serum (Fig. 7a, b). The MB time percentage variation effectively depicts the releasing behaviour of the substrates.

As expected, accordingly to the measured swelling degrees, the hydrogels are releasing more MB in the case of the NaCl solution (Fig. 7a) than in the case of the acid medium (Fig. 7b). The release of the model molecule follows a reciprocal relationship with the swelling behaviour [32]. In both cases however, the material synthesized with a 15 STMP:Xan ratio is having the lowest releasing rate (tangent on the percentage/time curves) and at equilibrium releases 3 (pH 1.5, Fig. 7b) to five times (NaCl, Fig. 7a) less MB by comparison to the gel with a crosslinking agent:polymer ratio of 10. The explanation of this behaviour is that a higher number of phosphate groups are physically binding a higher number of MB molecules hindering the consecutive releasing. Brannon–Peppas and Peppas [33, 34] analysed in detail the equilibrium and the dynamic swelling process as function of the pH of the swelling medium, and established the mechanism of the drug release from anionic, hydrophilic copolymers. In order to understand the mechanism of drug release from swellable polymers, researchers [35–37] sustain the proposal of the ionic interaction between ionizable drug and polymer with a reduction of the drug diffusion.

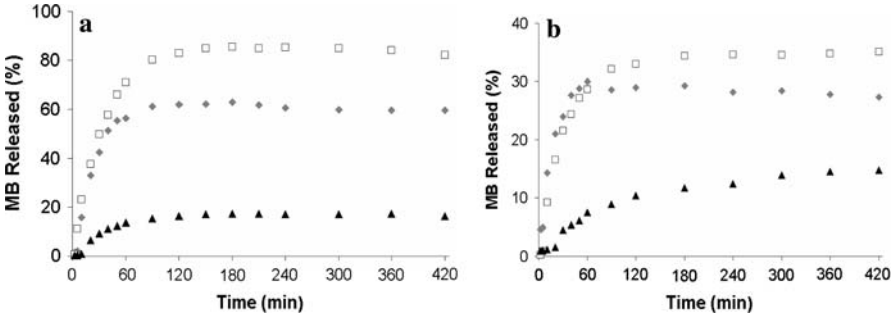


Fig. 7 Drug releasing behaviour: time variation of the MB percentage in the solution with respect to the initial quantity in the hydrogel: **a** NaCl 0.1 mol L^{-1}, **b** aqueous solution, pH 1.5. 25X5S (*rhombus*), 25X10S (*square*), 25X15S (*triangle*)

The highest releasing percentage was recorded for the 25X10S network. At equilibrium, after 180 min, it liberated in NaCl solution and in acid medium 85.5 and 34%, respectively, from the contained MB. According to the kinetics models presented above, the synthesized xanthan based systems present a first-order releasing kinetics.

Conclusions

In the present study, the obtaining of new STMP/xanthan networks based on a solvent free alkaline synthesis is being reported. Prior to crosslinking reaction, it was rheologically determined that in the reaction conditions the xanthan will be predominantly in its flexible, disordered coil form. The crosslinking agent/polymer ratio and the phosphate charges are oppositely acting in the three-dimensional network influencing the swelling behaviour. The balance between the two is responsible for the pH sensitiveness of the hydrogels. An intermediate STMP:Xan ratio lead to the highest swelling degree. An alkaline pH collapses the network by Na^+ cations screening of the phosphate charges with a considerable decrease in maximum swelling. The entrapping and releasing behaviour was examined using a cationic test molecule. Different releasing mechanisms in terms of rate and amplitude have been observed in function of STMP:Xan ratio and medium external parameters (pH, salt concentration) with a first-order kinetics. These are creating the premises of obtaining biocompatible pH and ionic strength sensitive substrates suitable for controlled drug delivery systems.

Acknowledgment This research was supported by the Région Haute Normandie (MILC grant).

References

1. Le Cerf D, Irinei F, Muller G (1990) Solution properties of gum exudates from *Sterculia urens* (karaya gum). Carbohydr Polym 13(4):375
2. Simon S, Picton L, Le Cerf D, Muller G (2005) Adsorption of amphiphilic polysaccharides onto polystyrene latex particles. Polymer 46(11):3700
3. Henni-Silhadi W, Deyme M, Boissonnade MM, Appel M, Le Cerf D, Picton L, Rosilio V (2007) Enhancement of the solubility and efficacy of poorly water-soluble drugs by hydrophobically-modified polysaccharide derivatives. Pharm Res 24(12):2317
4. Papagianni M, Psomas SK, Batsilas L (2001) Xanthan production by *Xanthomonas campestris* in batch cultures. Process Biochem 37:73
5. Martínez-Ruvalcaba A, Chornet E, Rodrigue D (2007) Viscoelastic properties of dispersed chitosan/xanthan hydrogels. Carbohydr Polym 67:586
6. Jansson PE, Kenne L, Lindberg B (1975) Structure of extracellular polysaccharide from *Xanthomonas campestris*. Carbohydr Res 45:275
7. Melton LD, Mindt L, Rees DA (1976) Covalent structure of the extracellular polysaccharide from *Xanthomonas campestris*: evidence from partial hydrolysis studies. Carbohydr Res 46:245
8. Moorhouse R, Walkinshaw MD, Arnott S (1977) Xanthan gum—molecular conformation and interactions. In: Sandford PA, Lasking A (eds) Extracelular microbial polysaccharides: a symposium. ACS Symposium series, Washington, p 90

9. Ogawa K, Yui T (1998) X-ray diffraction study of polysaccharides. In: Dumitriu S (ed) Polysaccharides: structural, diversity and functional versatility. Marcel Dekker, New York, p 101
10. Stokke BT, Elgsaeter A, Skjrak-Brjek G, Smidsrød O (1987) The molecular size and shape of xanthan, sylinan, bronchial mucin, alginate, and amylose as revealed by electron microscopy. Carbohydr Res 160:13
11. Capron I, Brigand G, Muller G (1997) About the native and renatured conformation of xanthan exopolysaccharide. Polymer 38:5289
12. Stokke BT, Christensen B, Smidsrod O (1998) Macromolecular properties of xanthan. In: Dumitriu S (ed) Polysaccharides. Structural diversity and functional versatility. Marcel Dekker, New York, p 433
13. Alupei IC, Popa M, Hamcerencu M, Abadie MJM (2002) Superabsorbant hydrogels based on xanthan and poly(vinyl alcohol) 1. The study of the swelling properties. Eur Polym J 38:2313
14. Mocanu G, Merle L, Carpov A, Muller G (2000) Immobilisation de biomolécules dans des microparticules de carboxyméthylcelluloses "hydrophobiquement modifiées". Eur Polym J 36:2381
15. Giri AK (1997) Genetic toxicology of epichlorohydrin: a review. Mutat Res Rev Mutat Res 386:25
16. Woo K, Seib PA (1997) Cross-linking of wheat starch and hydroxypropylated wheat starch in alkaline slurry with sodium trimetaphosphate. Carbohydr Polym 33:263
17. Kasemsuwan T, Bailey T, Jane J (1998) Preparation of clear noodles with mixtures of tapioca and high-amylose starches. Carbohydr Polym 32:301
18. Muhammad K, Hussin F, Ghazali YC, Kennedy JF (2000) Effect of pH on phosphorylation of sago starch. Carbohydr Polym 42:85
19. Gliko-Kabir I, Yagen B, Penhasi A, Rubinstein A (2000) Phosphated crosslinked guar for colon-specific drug delivery: I. Preparation and physicochemical character. J Control Release 63:121
20. Dulong V, Lack S, Le Cerf D, Picton L, Vannier JP, Muller G (2004) Hyaluronan-based hydrogels particles prepared by crosslinking with trisodium trimetaphosphate. Synthesis and characterization. Carbohydr Polym 57:1
21. Lack S, Dulong V, Le Cerf D, Picton L, Argillier JF, Muller G (2004) Hydrogels based on pullulan crosslinked with sodium trimetaphosphate (STMP): rheological study. Polym Bull 52:429
22. Mocanu G, Mihai D, Picton L, Le Cerf D, Muller G (2002) Associative pullulan gels and their interaction with biological active substances. J Control Release 83(1):41
23. Lack S, Dulong V, Picton L, Le Cerf D, Condamine E (2007) High-resolution nuclear magnetic resonance spectroscopy studies of polysaccharides crosslinked by sodium trimetaphosphate: a proposal for the reaction mechanism. Carbohydr Res 342:943
24. Sang Y, Prakash O, Seib PA (2007) Characterization of phosphorylated cross-linked resistant starch by 31P nuclear magnetic resonance (31P NMR) spectroscopy. Carbohydr Polym 67(2):201
25. Bejenariu A, Popa M, Le Cerf D, Picton L (2003) Stiffness xanthan hydrogels: synthesis, swelling characteristics and controlled release properties. Polym Bull 61:631
26. Hamcerencu M, Desbrieres J, Popa M, Khoukh A, Riess G (2007) New unsaturated derivatives of xanthan gum: synthesis and characterization. Polymer 48:1921
27. Arifin DY, Lee LY, Wang CH (2006) Mathematical modeling and simulation of drug release from microspheres: Implications to drug delivery systems. Adv Drug Deliv Rev 58(12–13):1274
28. Hadjiioannou TP, Koupparis CGD, Macheras MA, Panayotis E (1993) In: Quantitative calculations in pharmaceutical practice and research. VCH Weinhimi, New York, p 461
29. Bourne DWA (2002) Pharmacokinetics. Drugs and the pharmaceutical sciences. In: Banker GS, Rhodes CT (eds) Modern pharmaceutics, vol 121, 4th edn. Marcel Dekker, New York, p 67
30. Higuchi T (1963) Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 52(12):1145
31. Hixson AW, Crowell JH (1931) Dependence of reaction velocity upon surface and agitation. I. Theoretical consideration. J Ind Eng Chem 3:923
32. Talukdar MM, Kinget R (1995) Swelling and drug release behaviour of xanthan gum matrix tablets. Int J Pharm 120:63
33. Brannon-Peppas L, Peppas NA (1990) Equilibrium swelling behaviour of pH-sensitive hydrogels. Chem Eng Sci 46:715
34. Brannon-Peppas L, Peppas NA (1991) Time-dependent response of ionic polymer networks to pH and ionic strength changes. Int J Pharm 70:53
35. Bettini R, Colombo P, Peppas NA (1995) Solubility effects on drug transport through pH-sensitive, swelling-controlled release systems: transport of theophylline and metoclopramide monohydrochloride. J Control Release 37(1–2):105

36. Mocanu G, Mihaï D, Le Cerf D, Picton L, Muller G (2003) Synthesis of new associative gel microspheres from carboxymethyl pullulan and their interactions with lysozyme. Eur Polym J 40(2):283
37. Dulong V, Le Cerf D, Picton L, Muller G (2006) Carboxymethylpullulan hydrogels with a ionic and/or amphiphilic behavior: swelling properties and entrapment of cationic and/or hydrophobic molecules. Colloids Surf A 274:163