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Hydrogels Based on Pullulan Crosslinked with sodium trimetaphosphate (STMP): Rheological study

Stéphane Lack [1], Virginie Dulong [1], Didier Le Cerf [1]*, Luc Picton [1], Jean François Argillier [2] and Guy Muller [1]

[1] U.M.R. 6522, CNRS-Université de Rouen, F-76821 Mont-Saint-Aignan, France
[2] Institut Français du Pétrole (IFP), F-92852 Rueil-Malmaison, France

* Corresponding author: didier.lecerf@univ-rouen.fr (Didier Le Cerf); Fax: + 33 2 35 14 65 43

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ABSTRACT

Hydrogels from pullulan crosslinked with sodium trimetaphosphate (STMP) under alkaline condition have been investigated by rheological measurements. The influence of reagents concentration (pullulan, STMP, NaOH) on kinetics of reaction and rheological properties is reported. Increasing concentrations of any reagents lead to an increase of the crosslinking kinetics. Increasing the polymer concentration leads to a "stronger" gel whereas an increase of crosslinking reagent (STMP) evidences a critical concentration above which the rheological properties of the gel do not change anymore. This may be explained by the specificity of the system for which the polymer concentration is high and negative charges appear.

KEYWORDS: pullulan, STMP, polysaccharides, crosslinking, hydrogels.

1. Introduction

Hydrogels are three-dimensional networks made of crosslinked hydrophilic polymers. Crosslinks can result from physical [1] or chemical bondings [2,3]. Hydrogels are materials which, when placed in excess water, are able to swell and retain large volume of water in its swollen three dimensional structure without dissolution. This particular behaviour made them very attractive for many biomedical applications including contact lenses [4], time controlled drug delivery systems [5] but also for ion-exchange [6] separating membrane [7], and of course as superabsorbents [8].
The aim of this work was to study the elaboration of hydrogels. The starting material used as matrix in this work is pullulan, a linear and flexible extra cellular polysaccharide, which is biodegradable and biocompatible [9,10]. It structure consists of a linear chain of D-glucopyranosyl units that alternates regularly between one α(1-6) and two α(1-4) linkages. Sodium trimetaphosphate (STMP) has been chosen as a crosslinker, STMP is often used in food industry as it is non toxic for human [11] in contrast to epichlorydrin often used as crosslinker [12,13]. This paper concerns the

influence of the synthesis parameters (reagents concentration and temperature) on the crosslinking kinetics and on the rheological properties of such hydrogels. Measurements of the oscillatory shear moduli (mainly the storage modulus G') were used to monitor the viscoelastic properties of crosslinking during the gelling process, which were correlated with the evolution of the swelling behavior.

2. Materials and methods

2.1. Materials

Pullulan PI20 (Mn = 160 000 $g.mol^{-1}$), determined from SEC/MALLS measurements [14]) was purchased from Hayashibara Biochemical Laboratory (Okayama, Japan) and sodium trimetaphosphate from Sigma Chemical Company. Sodium hydroxide, sodium chloride and hydrogen chloride were purchased from WTR.

2.2. Hydrogel synthesis

The pullulan was dissolved in alkaline water (at fixed pH) [15] and stirred under gentle agitation for two hours. Then, an aqueous solution of STMP was added and the reaction mixture was stirred one minute [11,16]. Different concentrations of STMP, NaOH, polymer and temperature were tested. STMP and NaOH amounts were fixed respectively with $r_1 = \frac{[\text{NaOH}]}{[\text{AGU}]}$ and $r_2 = \frac{[\text{STMP}]}{[\text{AGU}]}$ ratio where (AGU) is the concentration of anhydroglucose units of pullulan. Adding an HCl solution (0.1N) until neutrality stopped the reaction. After neutralization, the gel was immersed in a large excess of water for at least two days. It is been verified that no macromolecule was released. This result shows that the percolation is reached. During this period, the water was replaced twice a day until reaching conductivity less than 5 µS. Hydrogels were deshydrated in acetone then dried at 45°C under vacuum. The dry hydrogels were kept in hermetically sealed vials at 6°C until further use. The gel was then characterized by the equilibrium swelling ratio Q in deionised water from Milli Q system (Millipore).

The mechanism of crosslinking using STMP is not precisely described in the literature, Gliko-Kabir *et al.* [16] propose the formation of an intermolecular phosphate link between two polymer chains. It is also possible to assume two other possibilities for the incorporation of phosphorus in the gel : STMP can react intramolecularly with two alcoholate belonging to the same polymer chain and/or with only one alcoholate group (monolinked phosphorus). In the two first cases each link affords one negative charge on the polymer whereas in the third two negative charges are brought on the chain. Elastically active chains only result from the first type of phosphorus incorporation.

The total amount of phosphorus atoms incorporated in the hydrogels was determined using elementary analysis. For all the gels prepared, 25 ± 3 % percent of the initial amount was always found. This low incorporation is due to degradation of STMP in alkaline media.[17].

2.3. Swelling test

A known weight of dry hydrogel was immersed in a vial containing a large excess of water at 24°C. The swollen hydrogel was removed after 24 hours and its excess surface water was wiped with paper until there were no visible water droplets. Gel swelling was quantified using the equilibrium weight swelling ratio Q, defined as:

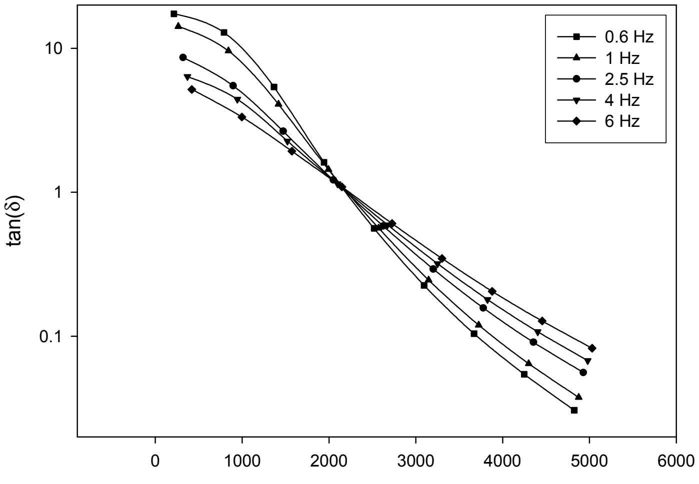
$$Q = \frac{weight \quad of \quad swollen \quad polymer \quad sample}{weight \quad of \quad dried \quad polymer \quad sample}$$

2.4. Rheological measurement

Rheology is a classical way to determine the gel point [18]. The gel point corresponds to a transition between a viscoelastic liquid characterized by its viscosity η and a viscoelastic solid exhibiting a large storage modulus G' as compared to the loss modulus G''[18].

The gel point was determined by following the evolution of tan (δ)=G''/G' versus time during the reaction for different oscillation frequencies (fig 1) [20]. The crossover of the plots gives the percolation time (t_{gel}). It is also interesting to note that the plateau value of G' is a relevant characterization of the complete and final structure of the studied gel.

Figure 1: Plots of tangent δ versus time. Each curve refers to a given frequency ω of the oscillary shear applied (0.6 to 6 Hz)



Rheological measurements were performed with an AR2000 rheometer from TA instrument using a cone/plate (2°, 4 cm radius cone) geometry. Concentrations of pullulan ranged from 150 $g.L^{-1}$ to 300 $g.L^{-1}$. Oscillation frequencies varied from 0.6 to 6 Hz and a suitable stress was applied for each concentration to be in the linear viscoelastic domain.

3. Results and discussion

3.1. Kinetic parameters

Study of the crosslinking kinetics is of primary importance for a better knowledge of the gelation. The influence of temperature, polymer, STMP and sodium hydroxide concentrations on the crosslinking kinetics was examined using rheology for the determination of the percolation time t_{gel}. The results are presented in table 1. All measures were reproducible.

Table 1: Percolation time as a function of synthesis parameters

	[pullulan] ($g.L^{-1}$)	$r_1 = \frac{[NaOH]}{[AGU]}$	$r_2 = \frac{[STMP]}{[AGU]}$	Temperature (°C)	t_{gel} (min)
reproducibility	200	0.15	0.22	24	30
	200	0.15	0.22	24	31
temperature	200	0.07	0.22	24	83
	200	0.07	0.22	45	22
[NaOH]	200	0.15	0.22	24	30
	200	0.07	0.22	24	83
[pullulan]	150	0.15	0.22	24	53
	200	0.15	0.22	24	30
	250	0.15	0.22	24	21
	300	0.15	0.22	24	12
[STMP]	200	0.15	0.22	24	30
	200	0.15	0.11	24	55

It appears that t_{gel} decreases upon increasing the temperature but also when the concentration of NaOH, STMP or pullulan increases. An elevation of the temperature should favour the mobility of STMP and polymer chains but also the chemical reactivity and, as a matter of fact, the crosslinking kinetics increases. The reactivity of pullulan involves the presence of alcoholate function, the proportion of which should increase when pH increases (i.e. with NaOH concentration). Obviously at higher concentrations of both polymer and crosslinking agent, the percolation time is reached faster than at lower concentration. This can be explained respectively by the fact that the polymer chains are closer (at higher polymer concentration) and by the increase of active species (at higher STMP concentration).

As it was expected the main parameters of the system largely influence the kinetics of the crosslinking reaction. Nevertheless it is a primary importance to study their influence on the final structure of the gel (i.e. the crosslink density).

3.2. Structural parameters

A way to evidence the influence of parameters on the hydrogels structure is to determine the storage modulus G' and the equilibrium swelling Q in water. Both parameters (G’ and Q) vary in reverse order, the swelling ratio Q decreasing with increasing G’ and vice versa.

3.2.1. *Influence of polymer concentration on crosslinking density*

Several hydrogels were synthesized which differ by the initial polymer concentrations, whereas temperature, NaOH and STMP concentration were fixed.

Figure 3: Plots of G’ (■), G’’ (◆) and Q (▼) versus pullulan concentration (T = 24°C, r_1 = 0.15, r_2 = 0.22, t = 48 H)

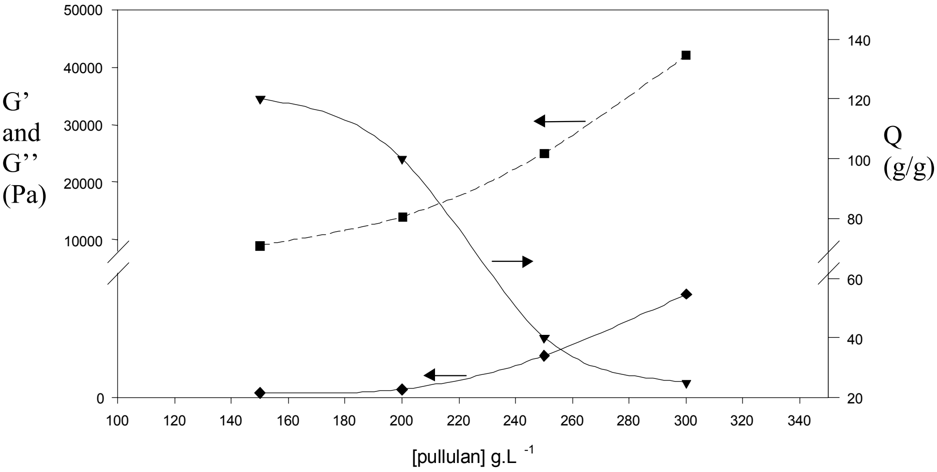


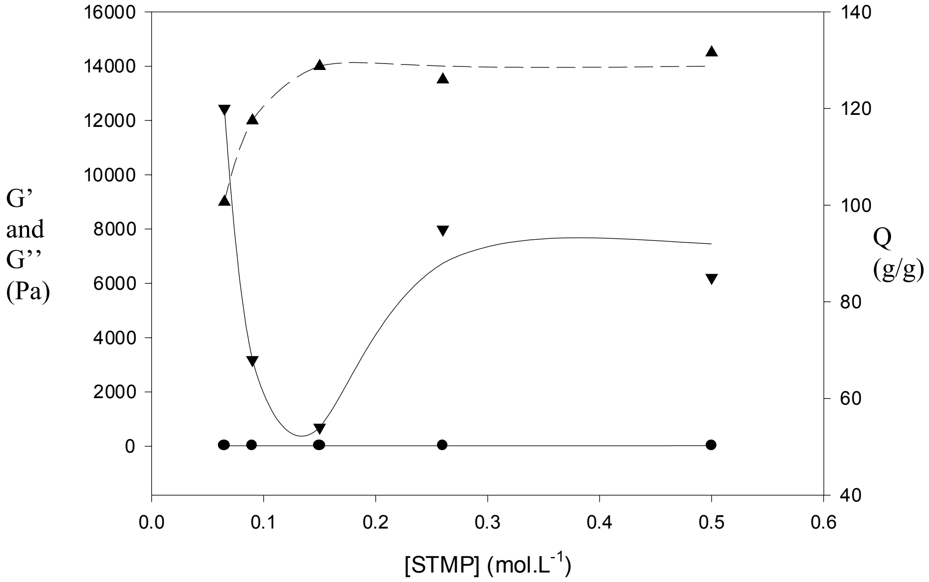
Fig 3 shows the effect of pulullan concentration on the storage and loss moduli G’ and G’’ and the swelling ratio Q for the different hydrogels : when the polymer concentration increases the ratio Q decreases and G’ and G’’ increase. This result means that the crosslink density increases as the pullulan concentration increases. Above a pullulan concentration of 250g.L^{-1}, Q reaches a constant value. At high polymer concentration rheology appears more sensitive than swelling ratio. The variation of both Q and G’ demonstrates that the mechanical properties depends on polymer concentration, which is not in agreement with what it was expected. The final structure should depend mainly on the number of chemical crosslinks i.e. the concentration of STMP. As the r_2 ratio has been kept constant in this experience, G’ should have remained constant.

In summary the initial polymer concentration increases the reaction kinetics but affects also the structure of hydrogels.

3.2.2. *Influence of STMP concentration on crosslinking density*

Five hydrogels have been synthesized differing by the STMP amount whereas the other parameters have been kept constant.

Figure 4: Plots of G' (▲) , G'' (●) and Q (▼) versus STMP concentration (T = 24°C, r_1 = 0.15, [pullulan] = 200 g.L^{-1}, t = 48 H)



On figure 4 are presented the variation of the elastic and loss moduli (G' and G'') and the swelling ratio (Q) as a function of STMP concentration. G' logically increases with the STMP concentration before a critical value of STMP concentration that can be evaluated around 0.15 mol.L^{-1}. This result evidences the increase of the cross-linking extent in agreement with the increase of the crosslinking reagent (i.e. STMP) concentration. In the same time, the swelling ration (Q) obviously decreases.

This behaviour is modified when the STMP concentration reaches the critical value of 0.15 mol.L^{-1}. Then above this critical crosslinking reagent concentration the elastic modulus does not change anymore, whereas at the same time a strong increase of the swelling ratio (Q) is observed up to a concentration of 0.3 mol.L^{-1} in STMP. One more time, this result does not agree with usual crosslinking rheological data. This result can be explained by the increase of charges brought by monolinked phosphorus that induces an increase of swelling by electric repulsions. This remark is reinforced by the fact that the yield of incorporated phosphorus appears quite constant (30%) when the STMP concentration increases (i.e. the amount of charges increases with STMP concentration). A hypothesis for explaining this behaviour will be proposed in the following discussion (3.2.4.).

3.2.3. Influence of NaOH concentration on crosslinking density

Three hydrogels were synthesized for different NaOH concentrations, all other parameters being kept constant.

Figure 5: Plots of G’ (▲) and Q (▼) versus NaOH concentration (r_2 = 0.22, [pullulan] = 200 g.L^{-1}, T = 24°C t = 48 H)

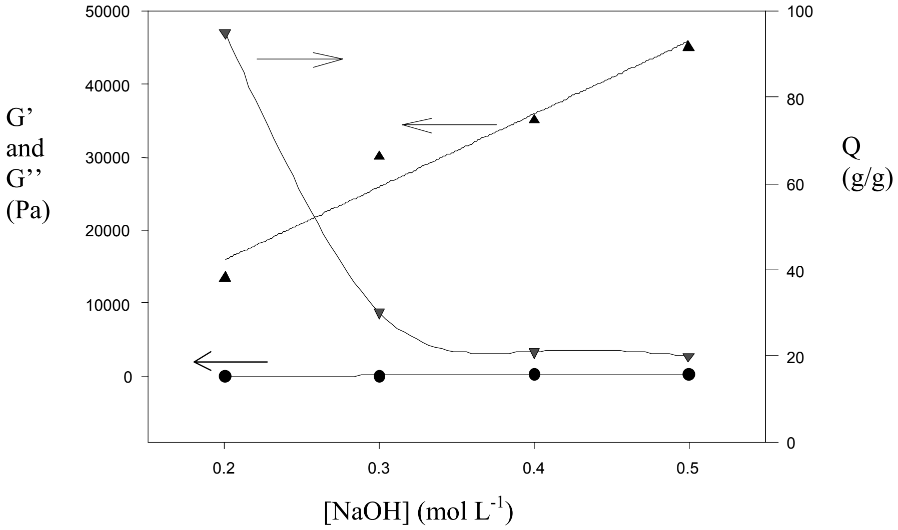


Fig 5 shows the evolution of G’ G’’ and Q for all samples versus [NaOH]. An increase of NaOH concentration induces a more crosslinked hydrogel with low Q and high G’. NaOH is added to the reactive blend in order to form alcoholates, which are needed to ensure the fixation of STMP. Our results evidence that a greater alcoholate concentration allows the formation of more crosslinking bridges. In summary, NaOH acts both on kinetics and structural factors.

3.2.4. Discussion

Rheological data concerning chemically crosslinked hydrogels generally show that the structure of the elaborated network characterized by a G’ value essentially depends on the crosslinking reagent concentration. The results we presented above do not agree with this picture. To find some explanation we can list the specific characteristics of our system.

First, the range of polymer concentration in our experiment (i.e. between 15% and 30%) is largely higher than that generally used for classical hydrogels systems (few percents) [16,21,22]. The density of chain entanglements is consequently very important in our case. The closeness of polymer chains may favour and influence the number of efficient links. Moreover the physical entangled network may contribute together with the chemically network to the final structure.

The second main characteristic of our system concerns the STMP and notably the incorporation of phosphorus on to the polymer via alcoholate groups. The literature is not abundant on this subject and no mechanism has been proposed so far. We can only suggest hypothesis that proposes three possibilities of phosphorus incorporation i.e. intermolecular (efficient link), intramolecular and monografted (inefficiency).

The differences between the pullulan/STMP system compared to other classical hydrogels may be explained by considering the effects of parameters such as [STMP], [pullulan], [NaOH] on the way of incorporation phosphorus particularly the competition between intermolecular phosphate link (two polymer chains) and monolinked phosphorus (one polymer chain). Moreover the incorporation of phosphorus bring negative charges that may also modulate both the yield and the kinetics of the reaction.

4. Conclusions

Hydrogels based on pullulan-STMP are attractive systems, which show specific characteristics.

The kinetics of crosslinking reaction is logically dependent on polymer, STMP, NaOH concentration and temperature. The final structure of such gels mainly depends on the polymer concentration but the crosslinking reagent (STMP) has a minor influence. Higher STMP concentration does not lead to a stronger gel above a critical amount of STMP. This result is consistent with the fact that the efficiency of crosslinking (intermolecular connection) is modulated by other parameters in competition that lead to intramolecular or monografted phosphorus.

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