proceedings

MDPI

Proceedings

Thermo/Shear-Responsive Injectable Hydrogels from an Alginate/PNIPAM-Based Graft Copolymer: Effect of Divalent Cations Ca^{2+} †

Sofia-Falia Saravanou, Fotoula Kounelaki and Constantinos Tsitsilianis *

Department of Chemical Engineering, University of Patras, 26500 Patras, Greece; faliasaravanou@hotmail.com (S.F.S.); fotkounel@gmail.com (F.K.)

* Correspondence: ct@chemeng.upatras.gr

† Presented at the First International Conference on "Green" Polymer Materials 2020, 5–25 November 2020; Available online: https://cgpm2020.sciforum.net/.

Published: 4 November 2020

Abstract: This work is focused on the design and development of biocompatible self-assembling hydrogels which behave as soft gels at room temperature and as strong ones at the physiological temperature, suitable for potential bio-applications. A graft copolymer of sodium-alginate bearing 8 side chains of poly(N-isopropylacrylamide), enriched with the hydrophobic comonomer N-tertiary-butyl-acrylamide (NtBAM), [NaALG-g-P(NIPAM-co-NtBAM)] were used as gelator. 5 wt% aqueous polymer solutions in the presence of Ca^{2+} cations were prepared and evaluated as thermoresponsive hydrogels. Rheological experiments revealed a twostep reversible gelation either upon heating or upon cooling. The divalent cations operate as cross-linking agent through ionic interactions inducing the formation of a network at low temperatures. Upon heating, an additional crosslinking develops through thermo-induced hydrophobic association of the thermo-responsive P(NIPAM-co-NtBAM) side chains above a critical temperature. The combination of thermo- and shear-responsiveness provides shelf-assembling systems as potential candidates for injectable strategies. For instance, the system under investigation could be used for cell transplantation, which require a weak gel to protect the cells during injection and a gel strengthening after the injection at physiological temperature to immobilize the created scaffold in the targeting position of the host tissue.

Keywords: alginate; P(Nisopropylacrilamide-co-N-tert-butylacrilamide); thermo/shear-responsive graft copolymer; divalent cations; injectability

1. Introduction

Hydrogels are constituted of three-dimensional networks highly swollen in aqueous media [1]. Hydrogels derived from natural polysaccharides attract great interest the last decades due to various potential applications in biomedical science, arising from their properties, such as biocompatibility, non-toxicity and biodegradability. The gelation capabilities of the polysaccharide macromolecules can be tuned using their functional groups through grafting strategies. For instance polysaccharides grafted with associative pendant chains (stickers) can form 3D networks with reversible crosslinks [2]. A very interesting category of hydrogels capable to respond to external stimuli, e.g., Temperature, pH etc., are those called "smart" hydrogels [3]. Provided that the network can be formed upon responding to a stimulus, injectable hydrogels can be designed, rendering them as potential candidate for drug and cell delivery systems.

Alginate hydrogels retain a structural similarity to the extracellular matrices in tissues and as a result these gels have promising applications in biomedicine and tissue engineering. Alginate is a natural and linear polysaccharide obtained from brown algae, consisting of (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) units [4]. Alginic acids as a negatively charged polymers exhibit the ability of gel formation via ionic interactions with divalent cations, such as Ca^{2+}, Mg^{2+} etc. [5]. The most common used cation is Ca^{2+}. The junctions between of Ca^{2+} and the carboxy groups of alginates are described as the egg-box model [6,7].

Thermo-responsive alginate-based graft copolymers have been developed recently. The hydrophilic backbone of alginate is grafted by the commonly used thermoresponsive polymer of N-isopropylacrilamide (PNIPAM), which exhibits a lower critical solution temperature (LCST) at about 32 °C, below the physiological one. This critical temperature is referred to the high molecular mass PNIPAM [8]. Hence, upon heating procedure hydrophobic associations of the thermoresponsive stickers occur above a critical gelation temperature (T_{gel}) and beyond a percolation concentration leading to a self-assembling network in water [3]. The reversible behaviour operates upon cooling. More importantly, the LCST can be tuned by enriching the thermoresponsive homopolymer chains with a comonomer [9]. The addition of a hydrophobic comonomer decreases the LCST to lower values and turns the sol-to-gel transition temperature. This effect influences all the rheological properties too and can be used to tune them at 37 °C (physiological conditions) [9].

The aim of this work was to explore the behaviour of a thermoresponsive alginate-based hydrogel in the presence of divalent cations Ca^{2+}. For this purpose, we use as gelator an alginate, grafted by 8 thermoresponsive side chains of poly(N-isopropylacrylamide), enriched with the hydrophobic comonomer N-tertiary-butyl-acrylamide (NtBAM). The main interest of the present work was to endow the thermoresponsive system with combined properties by adding Ca^{2+} ions as an additional cross-linking agent. Through the ionic interactions between the cations and the anions along the Na-Alginate backbone of the gelator a soft gel forms at lower temperature. Upon heating additional hydrophobic association of the side chains occurs. Overall, the system exhibits a soft to strong gel transition below and above the physiological temperature.

2. Experiments

2.1. Materials

The monomer N-isopropylacrylamide (NIPAM) and the co-monomer N-tert-butylacrylamide (NtBAM) were used as acquired by Fluorochem and Alfa Aesar, correspondingly. Potassium peroxodisulfate (KPS, Fluorochem) was utilized as initiator, 2-Aminoethanethiol hydrochloride (AET HCl, Alfa Aesar) was applied as chains transfer agent, 1-Ethyl-3-(3-(dimethylamino) propyl) carbodiimide (EDC, Alfa Aesar) and 1-Hydroxybenzotriazole hydrate (HOBt, Fluka) were used as coupling agents. Dimethylformamide (DMF, Aldrich), Hydrochloric Acid (HCl, Panreac) and Sodium Hydroxide (NaOH, Panreac) were used as obtained by the provider without purification. Sodium Alginate (NaALG, No. 180947, molecular weight range: 120,000–190,000 g/mol, the ratio of mannuronic and guluronic units (M/G): 1.53) was purchased in current state by Aldrich, within purification as reported by previous study [10].

2.2. Synthesis of the Graft Copolymer NaALG-g-P(NIPAM$_{94}$-co-NtBAM$_6$)-NH$_2$

The Grafting "onto" methodology was used to accomplish the synthesis of alginate-based graft copolymer. Briefly, $-NH_2$ end-functionalized P(NIPAM$_{94}$-co-NtBAM$_6$) random copolymers were grafted onto the $-COO^-$ groups of sodium alginate (NaALG) through carbodiimide chemistry. The resulted copolymer bears 8 thermo-responsive grafting chains. The molar ratio of the NIPAM/NtBAM monomers in the P(NIPAM$_{94}$-co-NtBAM$_6$) side chains is 94/6 (mol/mol) and the Mn 14,800 g/mol. The overall weight composition of the graft copolymer is 53 wt% NaAlg and 47 wt% P(NIPAM$_{94}$-co-NtBAM$_6$). Details of the synthesis and characterization are reported elsewhere [9].

2.3. Hydrogels Preparation

In order to produce solution with 4 mM concentration of Ca^{2+}, 0.0059 g of Calcium Chloride dihydrate ($CaCl_2$ H_2O) was dissolve into 10 mL distilled water and left under stirring in room temperature for 24 h. This solution was used as the aqueous media. Aqueous solutions of sodium alginate graft copolymer were prepared at a concentration of 5 wt%. After homogeneity, the pH of the solutions was regulated at the philological value of 7.4, using NaOH (1 M).

2.4. Rheological Studies

A stress-controlled AR-2000ex (TA Instruments) rheometer with a cone and plate geometry (diameter 20 mm, angle 3°, truncation 111 μm) was used to investigate the rheological properties of the sodium alginate graft copolymer aqueous solutions in terms of shear- and thermo-response. The experiments were accomplished in the linear viscoelastic regime, determined by strain sweep tests at frequency of 1 Hz. The samples were loaded at a Peltier plate system that ensure the experimental temperature with high accuracy (±0.1 °C). The rheometer was equipped with a solvent trap to prevent water evaporation during the experiments.

3. Results

To explore the thermo-indused properties of the NaALG-g-P($NIPAM_{94}$- co-$NtBAM_6$) aqueous solution in the presence of Ca^{2+} ions, rheological measuremnts were carried out through a temperature ramp oscillatory shear experiment. A heating/cooling cycles was accomplished with a rate of 1 °C/min. As it is shown in Figure 1a, the elastic modulus G′ predomonates the loss modulus G′′ in the entire temperature region, denoting the formation of a 3D network. At low temperatures, below the LCST of the side chains, the network formation is ascribed to the intermolecular ionic interactions arisen from the presence of Ca^{2+} ions (egg-box model). Upon heating and above a critical temperature (at about 30 °C) the moduli increase significally and a stronger network is formed due to the intermolecular hydrophobic association of the grafting side chains as an addition to the Ca^{2+} crosslinking. Importantly, both phenomena are reversible. In Figure 1b, tanδ is presented as afuncion of temperature. In all cases tanδ is lower than 1 confirming gelation. Moreover the hydrogel is strengthening upon increasing temperature as tanδ decreases steadily with temperature. We observe that the gel strenghtening is more pronounced above the critical temperature, due to the additional gelation arisen from the thermo- induced side chain association.

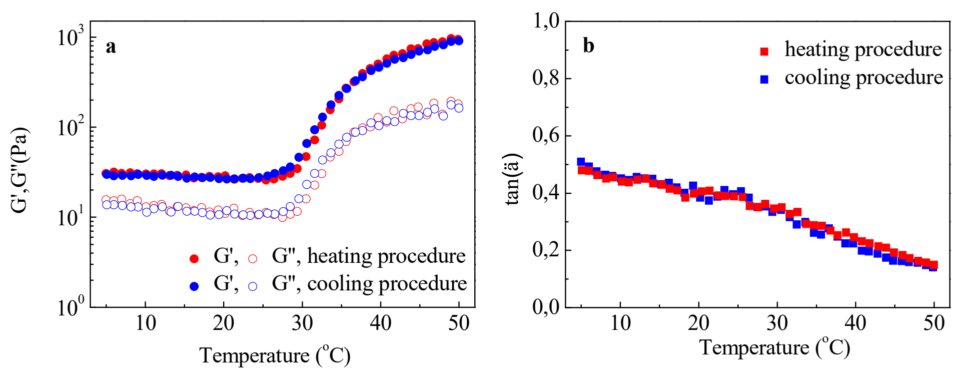

Figure 1. (**a**) Storage modulus G′ (solid symbols) and loss modulus G″ (open symbols) and (**b**) tanδ versus temperature in a heating/cooling cycle with a rate of 1 °C/min of a 5 wt% NaALG-g-P($NIPAM_{94}$-co-$NtBAM_6$) aqueous solution with 4 mM [Ca^{2+}] at pH 7.4.

Further studies were performed by oscillatory shear measurements at various constant temperatures. Plots of storage and loss modulus versus radial frequency are given in Figure 2. As it can be observed, the storage modulus is higher than the loss modulus in the frequency range investigated and the terminal relaxation zone is not visible in all investigated temperatures implying the formation of a 3D network. Moreover, the moduli increase with temperature in agreement with the temperature ramp data.

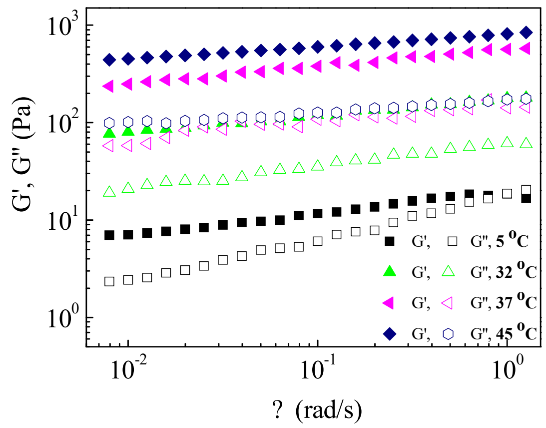

Figure 2. Storage G′ (solid symbols) and loss G″ (open symbols) moduli as a function of radial frequency of a 5 wt% NaALG-g-P(NIPAM$_{94}$-co-NtBAM$_6$) aqueous solution with 4 mM [Ca^{2+}] at pH 7.4.

The injectability of the hydrogel was evaluated, in terms of shear- and thermo-responsiveness, simulating experimental conditions similar to those of an injection through a 28-gauge syringe needle. By switching the shear rate from 0.01 s^{-1} to 17.25 s^{-1} at 20 °C (injection at room temperature) a remarkable shear-thinning effect was observed, as the viscosity decreased instantaneously about two orders of magnitude. Upon decreasing the shear rate at 0.01 s^{-1} and simultaneously increasing the temperature at 37 °C, (after injection at body temperature) the viscosity raised instantaneously three orders of magnitude. The viscosity is now higher than that at 20 °C more than one order of magnitude conforming the thermo-response of the system. By repeating the experiment the system showed excellent responsiveness and reversibility.

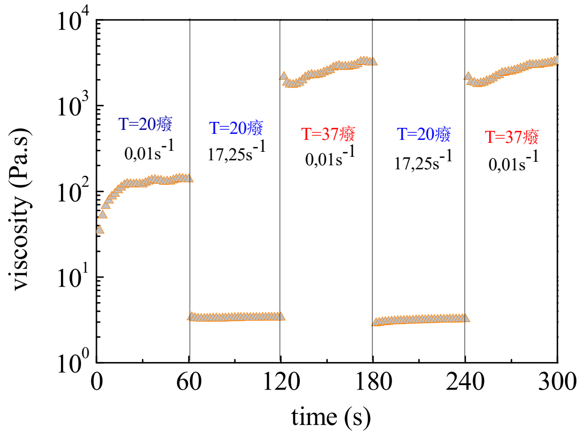

Figure 3. Shear viscosity versus time at different shear rates 0.01 s^{-1} (at 20 °C), 17.25 s^{-1} (at 20 °C), and 0.01 s^{-1} (at 37 °C) of a 5 wt% NaALG-g-P(NIPAM$_{94}$-co-NtBAM$_6$) aqueous solution with 4 mM [Ca^{2+}] at pH 7.4.

Finally, the self-healing of the system was explored by designing two consecutive experiments. A strain sweep test was firstly performed at room temperature beyond the linear viscoelastic regime. At high strains, G′′ becomes higher than the G′, as demostrated in Figure 4a, implying the destruction of the network. At Figure 4b a time sweep experiment was conducted with a strain value within the linear regime and the temperature at 37 °C. As seen, the network (hydrogel) is recovered almost instandaneously since the storage modulus prevails the loss one and at higher magnitudes due to thermoresponse. The initial retardation is due to the temperature equilibration on the instrument from 20 to 37 °C.

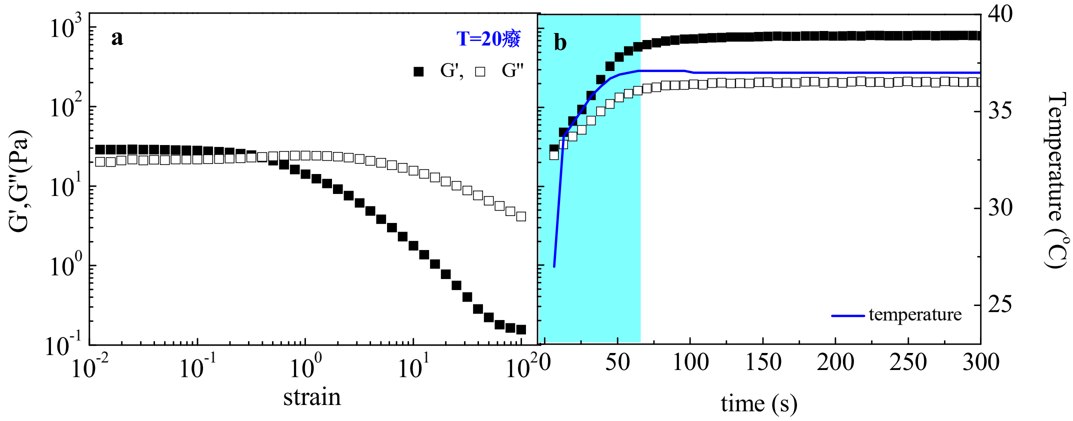

Figure 4. Strain sweep at 20° and consequent time sweep at 37 °C with applying strain within the linear viscoelastic regime of a 5 wt% NaALG-g-P($NIPAM_{94}$-co-$NtBAM_{6}$) aqueous solution with 4 mM [Ca^{2+}] at pH 7.4.

4. Conclusions

A 5 wt% aqueous polymer solution of a graft copolymer of sodium-alginate bearing 8 P(NIPAM-co-NtBAM) thermoresponsive side chains were investigated in the presence of 4 mM Ca^{2+} cations. The rheological data revealed a twostep gelation. At lower temperatures a soft gel is formed through ionic interactions between the divalent cation and the carboxyl anions of alginate. Upon heating a secondary hydrophobic crosslinking of the thermo-responsive side chains occurs leading to strong gel. Overall, the presence of Ca^{2+} transforms the behaviour of the system from a sol-to-gel transition (without Ca^{2+}) to a soft-to strong gel transition (with Ca^{2+}). The prepared hydrogel exhibits excellent injectability and self-healing induced by shear and temperature. These thermo/shear responsive shelf-assembling networks could be potential candidates for injectable strategies for stem cell transplantation This process requires a weak gel to protect the cells during injection and a stronger one after injection to immobilize the created scaffold in the targeting position of the host tissue.

Author Contributions: C.T. and S.-F.S. conceived and designed the experiments; F.K. performed the experiments; S.-F.S. and C.T. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

NaALG	Sodium Alginate
PNIPAM	Poly(N-isopropylacrylamide)
NtBAM	N-tertiary-butyl-acrylamide
NIPAM	N-isopropylacrylamide
KPS	Potassium Peroxodisulfate
AET HCl	2-Aminoethanethiol Hydrochloride
EDC	1-Ethyl-3-(3-(dimethylamino) propyl) Carbodiimide
HOBt	1-Hydroxybenzotriazole Hydrate
DMF	Dimethylformamide
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide
$CaCl_2$ H_2O	Calcium Chloride dihydrate

References

1. Peppas, N.A.; Bures, P.; Leobandung, W.; Ichikawa, H. Hydrogels in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27–46, doi:10.1016/S0939-6411(00)00090-4.
2. Leal, D.; de Borggraeve, W.; Encinas, M.V.; Matsuhiroa, B.; Müller, R.; Tsitsilianis, C. Preparation and characterization of hydrogels based on homopolymeric fractions of sodium alginate and PNIPAAm Responsive reversible hydrogels from associative ''smart'' macromolecules. *Carbohydr. Polym.* **2013**, *92*, 157–166, doi:10.1016/j.carbpol.2012.09.031.
3. Gomez, C.G.; Rinaudo, M.; Villar, M.A. Oxidation of sodium alginate and characterization of the oxidized derivatives. *Carbohydr. Polym.* **2007**, *67*, 296–304, doi:10.1016/j.carbpol.2006.05.025.
4. Yang, Y.; Campanella, O.H.; Hamaker, B.R.; Zhang, G.; Gu, Z. Rheological investigation of alginate chain interactions induced by concentrating calcium cations. *Food Hydrocoll.* **2013**, *30*, 26–32, doi:10.1016/j.foodhyd.2012.04.006.
5. Grant, G.T.; Morris, E.R.; Rees, D.A.; Smith, P.J.; Thom, D. Biological interactions between polysaccharides and divalent cations: The egg-box model. *FEBS Lett.* **1973**, *32*, 195–198, doi:10.1016/0014-5793(73)80770-7.
6. Sun, J.Y.; Zhao, X.; Illeperuma, W.R.K.; Chaudhuri, O.; Oh, K.H.; Mooney, D.J.; Vlassak, J.J.; Suo, Z. Highly stretchable and tough hydrogels. *Nature* **2012**, *489*, 133–136, doi:10.1038/nature11409.
7. Sun, J.Y.; Zhao, X.; Illeperuma, W.R.K.; Chaudhuri, O.; Oh, K.H.; Mooney, D.J.; Vlassak, J.J.; Suo, Z. Highly stretchable and tough hydrogels. *Nature* **2012**, *489*, 133–136, doi:10.1038/nature11409.
8. Pasparakis, G.; Tsitsilianis, C. LCST Polymers: Thermoresponsive Nanostructured Assemblies towards Bioapplications. *Polymer* **2020**, doi:10.1016/j.polymer.2020.123146.
9. Iatridi, Z.; Saravanou, S.F.; Tsitsilianis, C. Injectable self-assembling hydrogel from alginate grafted by P(Nisopropylacrylamide-co-N-tert-butylacrylamide) random copolymers. *Carbohydr. Polym.* **2019**, *219*, 344–352, doi:10.1016/j.carbpol.2019.05.045.
10. Ciocoiua, O.N.; Staikosa, G.; Vasile, C. Thermoresponsive behavior of sodium alginate grafted with poly(Nisopropylacrylamide) in aqueous media. *Carbohydr. Polym.* **2018**, *184*, 118–126, doi:10.1016/j.carbpol.2017.12.059.