



**UNICA**

UNIVERSITÀ  
DEGLI STUDI  
DI CAGLIARI



Università di Cagliari

**UNICA IRIS Institutional Research Information System**

**This is the Author's submitted manuscript version of the following contribution:**

Monduzzi M.\*, Musu G., Grosso M., Carucci C., Lindman B., Söderman O., Salis A.\*, Effect of electrolytes on the sol-gel phase transitions in a Pluronic F127/carboxymethyl cellulose aqueous system: phase map, rheology and NMR self-diffusion study, European Polymer Journal 181 (2022) 111707.

**The publisher's version is available at:**

<https://doi.org/10.1016/j.eurpolymj.2022.111707>

**When citing, please refer to the published version.**

This full text was downloaded from UNICA IRIS <https://iris.unica.it/>

# **Effect of electrolytes on the sol-gel phase transitions in a Pluronic F127/carboxymethyl cellulose aqueous system: phase map, rheology and NMR self-diffusion study.**

Maura Monduzzi,<sup>1,2,\*</sup> Giulia Musu,<sup>1</sup> Massimiliano Grosso,<sup>2,3</sup> Cristina Carucci,<sup>1,2</sup>

Björn Lindman,<sup>4,5</sup> Olle Söderman,<sup>4</sup> and Andrea Salis<sup>1,2,\*</sup>

<sup>1</sup> Dipartimento di Scienze Chimiche e Geologiche, University of Cagliari, SS 554, Bivio Sestu, 09042 Monserrato, CA, Italy;

<sup>2</sup> Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase (CSGI), via della Lastruccia 3, 50019 Sesto Fiorentino, FI, Italy;

<sup>3</sup>Dipartimento di Ingegneria Meccanica Chimica e dei Materiali, University of Cagliari, Via Marengo, 2, 09123 Cagliari, Italy;

<sup>4</sup> Division of Physical Chemistry, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden;

<sup>5</sup> Coimbra Chemistry Centre, Department of Chemistry, University of Coimbra, Rua Larga, 3004-535, Coimbra, Portugal.

\*Corresponding Authors:

Maura Monduzzi ([monduzzi@unica.it](mailto:monduzzi@unica.it))

Andrea Salis ([asalis@unica.it](mailto:asalis@unica.it))

**Keywords:** Pluronic F127, NaCMC, electrolytes, thermoresponsive hydrogels.

**Manuscript:** 5848 words (all captions and Tables are enclosed),

**Abstract:** 192 words

**Figures:** 5

**Tables:** 3

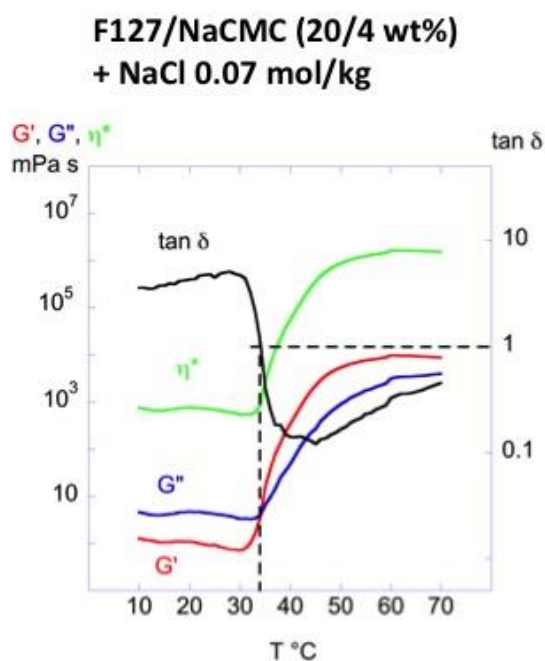
## **Abstract**

This investigation aims to obtain a thermosensitive platform useful for specific nanomedicine applications through the addition of suitable salts. An aqueous mixture consisting of the copolymer Pluronic F127 and sodium carboxymethyl-cellulose, in a ratio of 20/4 by weight, in the presence of different concentrations of  $\text{Na}_2\text{SO}_4$ ,  $\text{NaCl}$ , and  $\text{NaSCN}$ , was investigated using the visual inversion tube approach to estimate a phase map, and then characterized through rheological measurements. The formation of a cubic liquid crystalline phase at temperatures higher than  $37\text{ }^\circ\text{C}$  was suggested in all systems. The complex viscosity decreases in the presence of salts in the order  $\text{Na}_2\text{SO}_4 > \text{NaCl} > \text{NaSCN}$  with more marked effect for lower concentrations. For the polymer mixture and for a sample containing  $\text{NaCl}$ ,  $^1\text{H}$  NMR self-diffusion experiments display a non-mono-exponential decay of the echo signals of Pluronic F127, caused by the polydispersity of commercial Pluronic F127. The rheological properties along with the diffusion behaviour of the liquid crystal micelles suggest that a sample containing  $\text{NaCl}$   $0.07\text{ mol/kg}$ , with a sol-hard gel transition temperature around  $35\text{ }^\circ\text{C}$ , may be a suitable formulation for a potential thermosensitive drug delivery system.

## Graphical Abstract

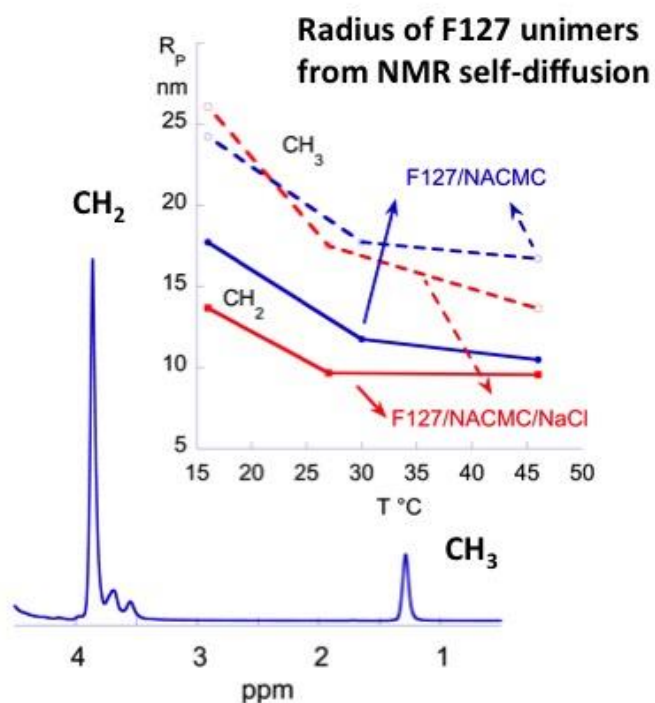
### Pluronic F127 / NaCMC Electrolytes effects

#### RHEOLOGY



#### NaCl

#### NMR PGSE



## Synopsis

A simple thermoresponsive platform for drug delivery: Pluronic F127, NaCMC and NaCl.

## Highlights

- ° Pluronic F127, NaCMC, and NaCl provide a thermoresponsive platform suitable for drug-delivery.
- ° Complex viscosity profile resembles the trend of the elastic modulus.
- ° Hofmeister effects are highlighted by complex viscosity.
- ° Salt addition decreases crowding effect of NaCMC on F127.
- ° PGSE NMR highlights F127 polydispersity.

## 1. Introduction

Water-based formulations generally contain both amphiphilic compounds, like surfactants and block copolymers, and hydrophilic polymers.[1,2] Whereas the amphiphilic compound traditionally was a simple low molecular weight surfactant, block copolymers, like Pluronics, ethylene oxide-propylene oxide copolymers have gained increased focus in recent years.[3–7] Regarding the hydrophilic polymers there has been a trend to go from synthetic ones to those based on polysaccharides;[8–13] polysaccharide-based polymers include cationic, anionic and nonionic ones.

Particularly, thermoresponsive copolymers are a class of "intelligent" materials that have the property of responding to stimuli due to changes in temperature, and can be functionalized to include other stimuli dependence such as pH, ionic strength, electric and/or magnetic fields and light.[14–20] These polymers have a wide range of applications including sensors, drug delivery or gene delivery systems and tissue engineering.[21–29]

Pluronic F-127 is a non-ionic three-block copolymer, namely  $EO_{97}PO_{68}EO_{97}$  (EO = oxyethylene, PO = oxypropylene), with relatively high molecular weight  $MW \approx 12.5$  kDa. The phase diagram, investigated by several authors,[30–34] shows that a clear solution of unimers and micelles exists at low temperature and concentration. At higher temperatures, phase transitions occur leading to the formation of liquid crystalline gels having different viscoelastic behaviour in response to changes either in temperature or in concentration. Some phase diagrams are reported in SI (Figures S1 and S2).[32,34] The formation of hard or soft gels is related to the occurrence of cubic liquid crystal (LC) phases.[31,35,36] Notably, a 20 wt% solution of highly purified Pluronic F127 is disordered for  $T < 13$  °C, shows face-centered cubic (fcc) structures LC in the range  $T = 13-22$  °C, and forms LC having body-centered cubic (bcc) structure for  $T > 22$ °C. For commercial Pluronic only face-centered cubic structures LC of slightly different nature were identified.[36]

Due to the thermoresponsive behaviour of Pluronics many investigations have reported rheological experiments to characterize the details of phase diagrams.[32,37–39] The addition of anionic or cationic surfactants to Pluronics leads to a lowering of the strength of the gel and to dissolution, due to the formation of mixed micelles.[40–43] Furthermore, the addition of large amounts of strong electrolytes can reduce the strength of the gel, thus modifying the phase diagram and the critical temperatures of sol-gel transitions and clouding.[44–47] These phenomena depend on the ability of the salt to influence the hydration energy of the PEO chain, although different and more complex phenomena are likely to be involved.[2,5,9,44]

Sodium carboxymethylcellulose (NaCMC) is another widely investigated polymeric hydrocolloid, obtained from cellulose treatment. NaCMC is a water-soluble anionic polyelectrolyte, often used as a modifier of the rheological properties, binding and thickening agent.[48–53] Furthermore, NaCMC can form films, is non-toxic, has a certain degree of adhesiveness, displays water retention characteristics and is resistant to oils, greases, and organic solvents. When monovalent salts are added in NaCMC solutions, the solution shows a behaviour typical of polyelectrolytes: the increase in the salt concentration leads to a decrease in electrostatic stiffening and "excluded volume" with a consequent decrease in the viscosity of the solution. [12,13,54,55]

Stability and dynamic behaviour of these macromolecules in aqueous solution can be modified by adding different salts, through electrostatic and dispersive interactions. Dispersive interactions are often referred as "Hofmeister effect",[56–59] and have been recognized in many chemical and biological processes through diverse technical approaches and modeling.[60–65] The solubility of macromolecules in water may decrease on adding anions in the order  $\text{HPO}_4^{2-} > \text{SO}_4^{2-} > \text{F}^- > \text{Cl}^- > \text{Br}^- > \text{I}^- > \text{NO}_3^- > \text{ClO}_4^- > \text{SCN}^-$ , or cations in the order  $\text{Cs}^+ > \text{NH}_4^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+ > \text{Mg}^{2+} > \text{Ca}^{2+}$ . It has been suggested that ions can somehow alter the structure of water and its macroscopic properties,[66–69] although a more intricate

phenomenon involving weak ion-polymer association is likely to occur when dealing with non-ionic polar polymers or polyelectrolytes. Indeed hydration of both ions and chains as well as direct interactions between the ions and the polymer backbone, involving a complicated balance between electrostatic and dispersion forces, should be considered,[5,58,70–73] For instance, the “solute partitioning model” by Pergram and Record [74–76] suggests that the “salting-out” effect is due to the exclusion of ions from the hydration sphere of the polymer and that the “salting-in” effect is due to the accumulation of ions at the polymer interface. Interestingly, the amount of each salt to produce Hofmeister effects is significantly dependent on the type of system as widely demonstrated.[72,77–79]

In this study we consider a mixed system of an EO-PO block copolymer, Pluronic F 127, and an anionic cellulose derivative, sodium carboxymethyl cellulose, NaCMC.[10–13] In such a system, interactions with ions will be crucial but the two components will respond very differently. The Pluronic will, as clearly demonstrated in phase separation studies,[40,44,46,47,80] associate with polarizable ions to increase the temperature of phase separation from water (the cloud point); ions with low polarizability will show an opposite effect and decrease the solubility of the Pluronic. For a polyelectrolyte like NaCMC, the interaction with electrolyte will be completely different and follow another sequence. To understand the behaviour, crucial for formulation work, we studied the effect of different electrolytes on a mixed system of Pluronic F127 and NaCMC, a typical combination in many formulations. Pluronic shows a self-assembly which is strongly dependent on temperature, leading to phase changes, whereas NaCMC is largely independent on temperature. The main aim is to obtain tunable phase transition temperatures through salt addition for a thermosensitive formulation platform potentially useful for specific nanomedicine applications.

Most investigations related to salt addition to thermosensitive polymers imply the use of salt concentrations higher than 1 M, whereas the present work focuses on the use of much lower

salt concentrations to highlight specific subtle interactions between the polymer mixture and the hydrated ions while minimizing the role of ionic strength. Here, we report a qualitative phase map obtained through the visual inversion tube method, the sol-gel transition temperatures in some selected samples determined using rheological measurements,[39,81] and molecular motions highlighted by NMR self-diffusion data.[82–88]

## **2. Experimental**

See SI for used chemicals and technical details (Sample preparation, inversion tube, rheological measurements and NMR self-diffusion).

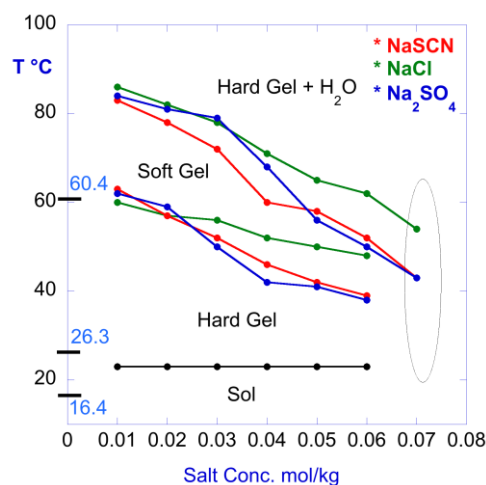
## **3. Results and Discussion**

### **3.1 Sol-gel phase maps.**

The samples containing a fixed ratio of Pluronic F127/NaCMC = 20/4 w/w (the weight fraction of F127 was 20 wt% in all samples) and sodium salts at the concentrations 0.01-0.07 mol/kg (referred to the solvent amount) were investigated to obtain a preliminary evaluation of phase transition temperatures through the inversion tube method.

Figure 1 shows the heating curves for the examined samples. See also Figures S3 a-c in SI. The first sol-gel transition is observed at about 23 °C for all samples in the range of salt concentration 0.01-0.06 mol/kg. According to literature, this gel can be defined as a hard gel, consisting of micelles organized in compact fcc cubic liquid crystals.[36]





**Figure 1.** Inversion tube results. All samples contain 24 wt% of polymer mixture (F127/NaCMC = 20/4), and salt concentration is referred to solvent content (76 wt%). Temperatures (light blue) on the T axis indicate data observed for F127 alone, from DSC experiments, as cmt (16.4 °C), sol-hard gel (26.3 °C), and hard gel-soft gel (60.4°C) transitions (see Figure S1 in SI).[32]

In agreement with findings for F127 alone three phase transitions are observed: 1) sol-hard gel, which is a transition from micellar solution to cubic phase; 2) hard gel-soft gel which is a transition between two cubic phases, “softness” being induced by shrinking EO chains; 3) separation into two phases, one polymer-rich and one water-rich, i. e. a typical clouding phenomenon. Strikingly, transition 1) occurs at lower temperature than that found for F127 alone but is apparently unaffected by electrolyte concentration and there is no difference between the different electrolytes. Regarding transitions 2) and 3) there are qualitatively the same effects for the different electrolytes with some quantitative differences in the presence of NaCl, with increasing salt concentration. In the case of a salt concentration of 0.07 mol/kg samples were fluid solutions up to about 43 and 44 °C in the presence of Na<sub>2</sub>SO<sub>4</sub> and of NaSCN, and up to about 54 °C in the presence of NaCl. The temperatures at which these transitions occur decrease with increasing salt concentrations. Clouding temperature, however, cannot clearly be identified through visual inversion tube method. For salt concentration 0.07 mol/kg, samples show only one sol-hard gel transition: a further increase of temperature induced a weak turbidity but no significant increase of fluidity. In summary, the visual measurements

carried out with the inversion tube method show that the addition of salts to the F127-NaCMC system shows similar transition temperatures as found for the two sulfate and thiocyanate anions that are at opposite ends of the Hofmeister series. The chloride, on the other hand, differs considerably from the trends observed for the other two anions.

Another crucial point, mentioned in the experimental section, is related to the fact that thermoreversibility in cooling ramps occurs in very long times compared to heating ramps (cfr. Figures S3 a-c in SI). Indeed, clouding implies phase separation whose consequence is the formation of very small water droplets that, during cooling, the liquid crystal gel can reabsorb only very slowly thus determining a marked hysteresis, namely an apparent non-thermoreversibility. Very long cooling times to rearrange the polymer chains of F127, intermingled with those of NaCMC, are needed. Similar behaviour was observed also for rheology experiments (see below).

An analysis of the thermal behaviour of the aqueous system of F127-NaCMC-electrolyte must be based on the understanding of the different systems with two cosolutes, namely the polyelectrolyte NaCMC and salt in water. Electrolyte addition to aqueous solutions of nonionic surfactants and block and graft copolymers with EO groups as well as of many other nonionic polymers, like cellulose derivatives, give a consistent picture: highly polarizable anions like iodide and thiocyanate favour the compatibility with water whereas small less polarizable ions give the opposite effect. The simplest manifestation of this is that the former ions raise the cloud point whereas the latter ones lower it. The underlying mechanism as well as other consequences have been described in several publications. In the case of F127 we can note that polarizable ions such as  $\text{SCN}^-$  raise the temperatures of the different transitions whereas the opposite is true for small ions such as  $\text{Cl}^-$ .<sup>[44]</sup> Both cmt and Cloud Point decrease in the presence of NaCl and increase when adding NaSCN.<sup>[47]</sup> However, in all cases, the effects are never significant for salt concentrations lower than 0.2 M.<sup>[44,47]</sup> Notably, the addition of

Na<sub>2</sub>SO<sub>4</sub> (0.28 molal) was shown to cause the loss of gel formation in a solution of F127 20 wt%.<sup>[46]</sup>

Adding a hydrophilic polymer that does not associate with F127 is expected to have a “crowding” effect, effectively increasing the F127 concentration and thus shifting the thermal transitions to lower temperatures. Since NaCMC has high charge density, general electrostatic effects rather than polarizability dominate. On salt addition NaCMC contracts, and the effective volume of the polyion in water decreases leading to a reduction of the crowding effect. As can be seen from the results (see also below in rheology section) the different expectations are confirmed in our study, including a lowering of the sol-gel transition by NaCMC, and a lowering of both the hard gel-soft gel and clouding temperature by electrolyte addition.

### **3.2. Rheological measurements**

Two samples without salt, one containing F127 20 wt% (sample F), and the other the mixture F127/NaCMC = 20/4 w/w (sample F/C), along with samples F/C containing 0.05 and 0.07 mol/kg of salts were selected for rheological measurements. Table 1, in the 1<sup>st</sup> column, reports name and composition of the investigated samples. Temperature ramps with a heating rate of 1°C min<sup>-1</sup>, and oscillatory frequency of 1 Hz were carried out.

The trends of the parameters  $G'$  (storage modulus),  $G''$  (loss modulus),  $\tan \delta$  (loss factor) and complex viscosity  $\eta^*$ , described in Eq.s S1-S3, were examined.

**Table 1** Rheological parameters obtained from the increasing temperature ramps.

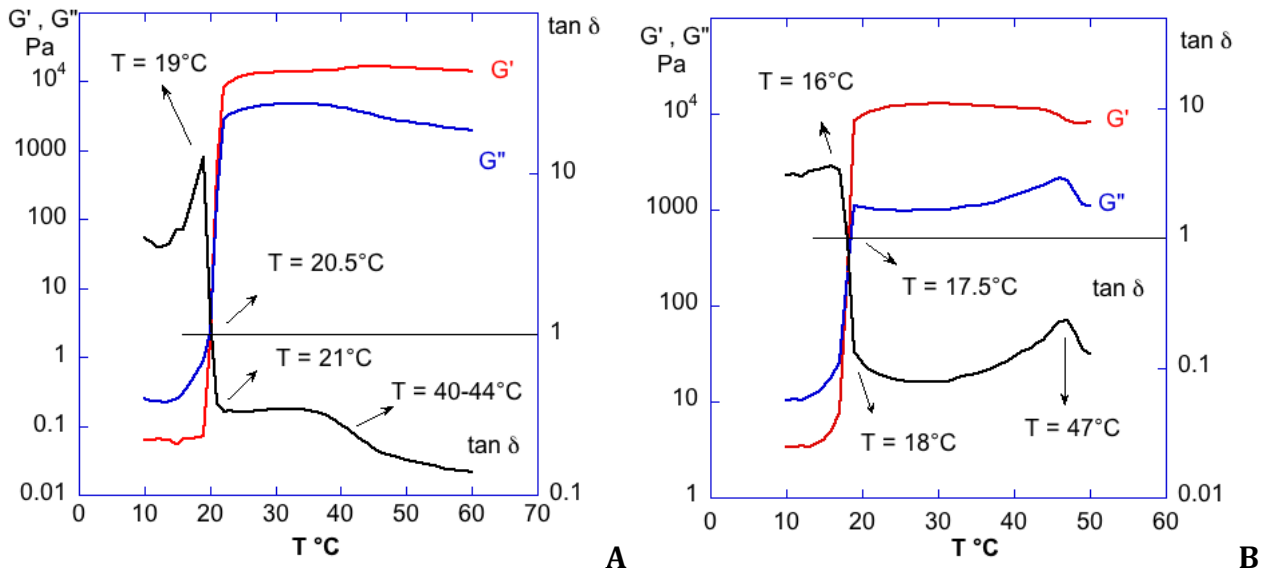
<b>Sample</b> (salt conc. in mol/kg)	<b>T °C</b> <b>(cmt)</b>	<b>tan <math>\delta</math> (T °C)</b> <b>Max</b>	<b>T °C</b> <b>(sol-hard gel)</b>	<b><math>\eta^*</math> (T °C)</b> <b>(tan <math>\delta &gt; 1</math>)</b> <b>mPa s</b>	<b><math>\eta^*</math> (T °C)</b> <b>(tan <math>\delta &lt; 1</math>)</b> <b>mPa s</b>
F Pluronic F127	19.0	12.5	20.5	448 (20)	91736 (21)
F/C F127+NaCMC	16	3.66	17.5	4231 (17)	49296 (18)
F/C_1 + Na <sub>2</sub> SO <sub>4</sub> 0.05	20	4.79	25.5	3732 (25)	6568 (26)
F/C_2 + Na <sub>2</sub> SO <sub>4</sub> 0.07	20	11.1	25.5	1878 (25)	19719 (26)
F/C_3 + NaCl 0.05	15	4.44	21.5	3404 (21)	5408 (22)
F/C_4 + NaCl 0.07	28	5.09	34.5	869 (34)	2848 (35)
F/C_5 + NaSCN 0.05	22	6.37	27.5	2088 (27)	5662 (28)
F/C_6 + NaSCN 0.07	23	4.31	24.5	745 (24)	1863 (25)

Table 1 reports the following data: the cmt (column 2) identified as the T at which the highest value of  $\tan \delta$  (column 3) is measured before the sol-gel transition; the sol-hard gel transition T (column 4) is identified at  $\tan \delta = 1$ , when  $G' = G''$ ; the complex viscosity  $\eta^*$  measured at the lowest T before ( $\tan \delta > 1$ , column 5) and after the sol-gel transition ( $\tan \delta < 1$ , column 6). Figure 2 shows  $G'$ ,  $G''$  and  $\tan \delta$  for samples F (3A) and F/C (3B), whereas data for samples containing salts are reported in SI, Figure S4 (a-f). The (hard gel)–(soft gel) transition was not clearly identified due to the complexity of the  $G'$  and  $G''$  trend in the presence of salts 0.05 mol/kg (see Figures S3 in SI).

### 3.2.1 Effect of NaCMC addition to Pluronic F127

To evaluate the effect of adding NaCMC we note that the main differences in the parameters shown in Table 1 and Figure 2 are associated with the transition temperatures and to the values

of the moduli and of  $\tan \delta$ , particularly for  $T > 30$  °C.



**Figure 2.** Rheology experiments. Storage Modulus ( $G'$ ), and Loss Modulus ( $G''$ ) on left axis (log scale) and Loss Factor ( $\tan \delta = G''/G'$ ) on right axis (log scale) vs.  $T$  (°C) for **(A)** Pluronic F127 20 wt% (sample F); **(B)** Pluronic F127 20 wt% + NaCMC 4 wt% (sample F/C).

Both cmt and sol-hard gel transition temperatures decrease in the presence of NaCMC. The formation of a hard gel is related to the assembly of micelles in highly viscous cubic liquid crystals, likely with fcc structure.[31,35,36,89] The formation of the gel implies that the elastic component becomes more important, that is  $G' > G''$ , and  $\tan \delta < 1$ . In the case of F127  $\tan \delta$  does not change significantly in the temperature range 21-40 °C. With further increase of  $T$ ,  $G'$  increases while  $G''$  decreases, thus  $\tan \delta$  decreases, likely due to the (hard gel)-(soft gel) transition for  $T > 50$  °C. In the presence of NaCMC, after the sol-hard gel transition,  $\tan \delta$  fluctuates and increases up to 47 °C: this maximum can tentatively be associated to the starting of the (hard gel)-(soft gel) transition. This is in line with our interpretation above on a crowding effect on F127 exerted by NaCMC leading to a lowering of the temperature for micelle and cubic liquid crystal formation. Finally, we can remark that the complex viscosity  $\eta^*$  (see Table 1, columns 5 and 6) just before the sol-gel transition increases almost one order of magnitude

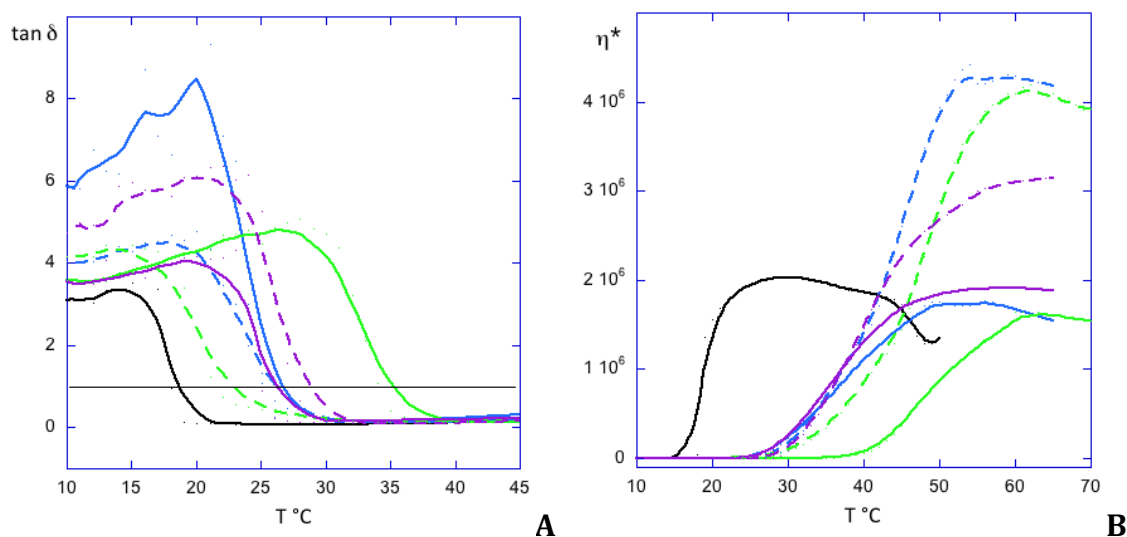
because of NaCMC addition, while it decreases by about a factor of two immediately after the transition into the gel phase.

This behaviour indicates quite complex interactions between the non-ionic Pluronic polymer and the polyelectrolyte NaCMC as a result of a dominant crowding effect along with a rebalancing between hydration and electrostatic forces.

### 3.2.2 Effect of salt addition to F127/NaCMC mixture

Considering the rheological data, obtained from the parameters shown in SI (Figures S3) and reported in Table 1, we note that salt addition increases both the temperatures of micelle formation ( $T_m$ ) and of the sol-(hard gel) transition. In addition, we highlight the following trends as a function of salts and their concentration: i)  $\tan \delta = 1$  is observed at increased  $T$  in the order  $\text{Cl}^- < \text{SO}_4^{2-} < \text{SCN}^-$  for salts 0.05 mol/kg, and  $\text{SCN}^- \leq \text{SO}_4^{2-} < \text{Cl}^-$  for salts 0.07 mol/kg; ii)  $\eta^*$  in the vicinity of the sol-gel transition is always lower than that determined in the absence of salts (see Table 1, columns 5 and 6), likely as a consequence of the higher transition temperatures.

The trend of  $\tan \delta$  vs.  $T$  reflects the variations of the balance between the viscous and the elastic behaviour (see eq. S2) and allows to clearly detect the sol-gel transition temperature. Similarly, the complex viscosity, calculated through eq. S3, is a measure of the total resistance to flow as a function of  $T$  at constant oscillation frequency (1 Hz). Notably, these features play a crucial role in addressing stability and possible applications of a formulation platform. This can clearly be seen in Figure 3 where  $\tan \delta$  vs.  $T$  (Figure 3A) and  $\eta^*$  vs.  $T$  (Figure 3B) for the polymer mixture F/C alone (black line) and in the presence of the three salts at the concentration of 0.05 mol/kg (dashed lines) and 0.07 mol/kg (continuous lines) are reported.



**Figure 3.** Effect of salts on rheological behaviour. **(A)**  $\tan \delta$  vs.  $T$  (10-45  $^{\circ}\text{C}$ ) and **(B)** Complex Viscosity  $\eta^*$  vs.  $T$  (10-70  $^{\circ}\text{C}$ ) for sample F/C alone (black line), and in the presence of salts Na<sub>2</sub>SO<sub>4</sub> (blue), NaCl (green), NaSCN (violet) at different concentrations: dashed lines 0.05 mol/kg; and continuous lines 0.07 mol/kg. The  $\tan \delta$  and  $\eta^*$  are here shown after a smoothing treatment (all original data are reported in SI Figures S4 and S6).

Substantially,  $\tan \delta$  shows a much less steep decrease during the sol-(hard gel) transition than that observed for samples F and F/C (see Figure 2), particularly in the presence of salts 0.05 mol/kg when the different sol-gel transitions occur through a multi-step process within a large range of temperature. These trends agree with the transitions observed through the inversion tube method. The trend of complex viscosity  $\eta^*$ , shown in Figure 3B, highlights the significant decrease of the values due to the increase of salt concentration. Both  $\tan \delta$  and  $\eta^*$  indicate a significant increase of the temperature for the sol-(hard gel) transition, particularly in the presence of NaCl 0.07 mol/kg.

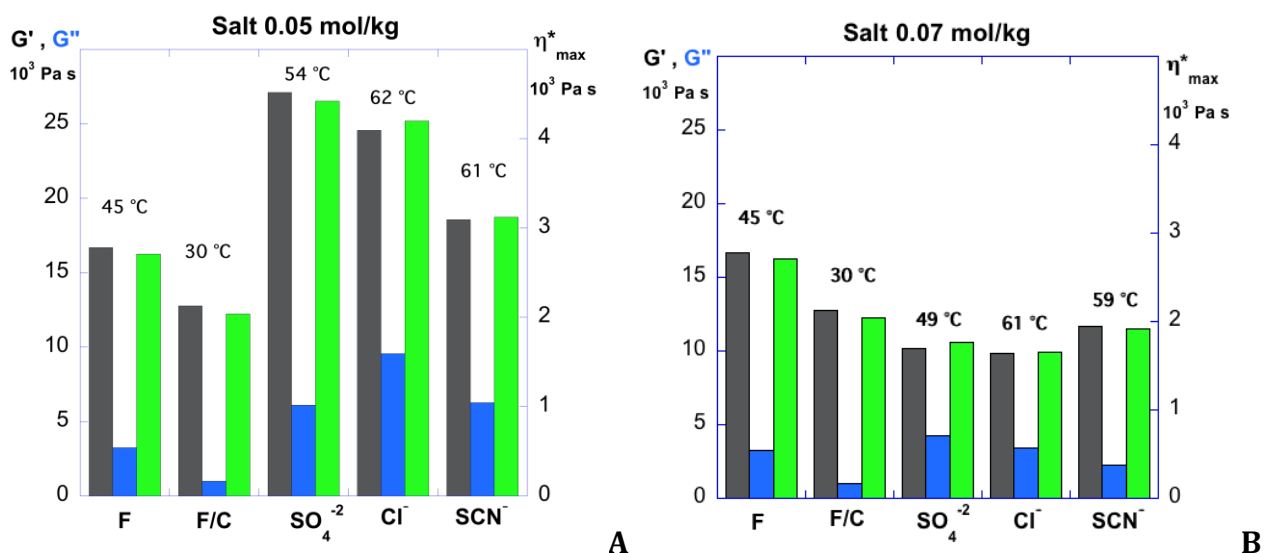
Before commenting on these data, we should recall that an increase of  $\tan \delta$  is generally due to an increase of energy dissipation potential (viscous behaviour), whereas a decrease is due to an increase of energy storing capability (elastic behaviour), that can simply be ascribed to the presence of objects and interactions that impose restrictions to molecular motions.

Several works have reported that Pluronic polymers, polyelectrolytes and their mixtures are strongly affected by cosolute addition as far as rheological behaviour[40,44,47,90] and other physical properties, such as cmt, melting and clouding points, solubility, surface tension, or intrinsic viscosity[12,50,77,91–96] are concerned. In several cases, anions showed a clear Hofmeister effect on cmt and clouding, that is, kosmotropic anions decrease whereas the chaotropic anions increase the transition temperatures.[41,44,47,77,90,94] Substantially, it has been pointed out that several added salts decrease viscosity of NaCMC solutions, due to a decreased electrostatic stiffening and excluded volume.[12,97] In the case of Pluronic F127 (30 wt%), the addition of small amounts of NaCl caused a decrease of the storage modulus.[93] In addition, some works based on light scattering experiments by Zhang et al., [72,77,94,98] highlighted complex mechanisms related to phase transition temperatures of some polymers (namely Pluronic L44 and PNIPAM) as a function of temperature, in the presence of sodium salts at different concentrations: poorly hydrated anions modulate temperature transitions through a two-step mechanism, and interact specifically with polymer hydrophobic groups, whereas highly hydrated anions, due to significant interaction with polymer hydrophilic moieties, cause entropic changes in the polymer hydration water molecules, and modulate temperature transition via a single step. Presumably, the simultaneous presence of two kinds of polymers, one of which is a polyelectrolyte sharing the sodium cation with the added salts, may further complicate the mechanisms underlying the phase transitions.

Indeed, turning the attention to our results, we can notice that the trends of  $\tan \delta$  and  $\eta^*$  in Table 1 and Figure 3 do not allow an unequivocal interpretation of salts effects in terms of concentration or Hofmeister series. However, focusing on the complex viscosity, we can note that the storage modulus  $G'$  addresses substantially the trend of  $\eta^*$  as a function of temperature. Indeed, the highest values of both  $\eta^*$  and  $G'$  occur at the same temperature in each system (see the plots of  $G'$ ,  $G''$  and  $\eta^*$  for all systems, reported in SI Figure S6 b-h, where the same linear



scale was adopted to allow for a comparison among the different systems and on the effect of concentration for each salt). Figure 4 summarizes the main results obtained in the presence of salts 0.05 (Figure 4A) and 0.07 (Figure 4B) mol/kg, presented in comparison with samples F and F/C without salts.



**Figure 4.**  $G'$  (dark grey bars),  $G''$  (blue bars), and  $\eta^*$  (green bars), in the absence and in the presence of different sodium salts 0.05 mol/kg (A) and 0.07 mol/kg (B). Data for samples F and F/C without salts are given for comparison.

A clear effect of salt addition can easily be seen considering the values of the  $G'$  and  $G''$  after the sol-(hard gel) transition: i)  $G' > G''$  is always observed; ii) both  $G'$  and  $G''$  along with  $\eta^*$  show a significant decrease with increasing salt concentration (see the different y-scales in Figures 4A and 4B); iii) the maximum viscosity value  $\eta_{\text{max}}^*$  of each system is recorded at different T, but, notably, decreases with increasing salt concentration, as also observed for  $\eta^*$  immediately before and after the sol-gel transition (see Table 1, column 5 and 6). The marked decrease of  $\eta^*$  with increasing salt concentration is clearly seen also in Figure 4B.

Data obtained for samples F and F/C, also reported for comparison, highlight an interesting result, that is the addition of NaCMC significantly decreases either the T at which  $\eta_{\max}^*$  is observed ( $\approx 15^\circ\text{C}$ ), or the values of  $G'$  ( $\approx 23\%$ ),  $G''$  ( $\approx 69\%$ ), and  $\eta_{\max}^*$  ( $\approx 25\%$ ).

Salt addition causes a significant increase in the temperature at which  $\eta_{\max}^*$  is observed, and for each salt, the increase of concentration causes a decrease of all the parameters. Notably, the anion Hofmeister series can clearly be seen in the difference between the two concentrations (0.05-0.07) for the parameters  $G'$  and  $\eta_{\max}^*$ , but not for  $G''$  (see Table S1 in SI): the difference between the two concentrations decreases in the order  $\text{SO}_4^{2-} > \text{Cl}^- > \text{SCN}^-$ . This indicates that increasing the concentration of a weakly hydrated anion, such as  $\text{SCN}^-$ , the storage modulus and hence complex viscosity are less affected than in the case of a well hydrated anion such as  $\text{SO}_4^{2-}$ . In summary, however, we should remark that the  $\text{Cl}^-$ , that is located in the middle of the Hofmeister series, is the anion that, at the relatively small concentration of 0.07 mol/kg, induces a very high increase ( $17^\circ\text{C}$ ) in the sol-(hard gel) transition temperature.

The complexity of the system makes macroscopic behaviour, like rheology, dependent on many molecular interactions. The rheological data demonstrate that the system is quite sensitive also to relatively minor changes in either composition or the identity of the electrolyte added; they are in line with the phase transition picture described above. A general feature is that NaCMC is sensitive to electrolyte addition. The crowding effect leads to an effective concentration increase of F127 that is counteracted on electrolyte addition. The effect of electrolyte addition on NaCMC is only weakly specific, whereas for F127 a marked anion specificity has been detected,[44,46,47] using however relatively high salt concentrations. The balance of these effects underlies the rich rheological behaviour as discussed above.

### 3.3 NMR self-diffusion experiments

To extract information on the dynamics of the F127 as a function of temperature, F/C and F/C\_4 samples were selected for NMR PFG experiments to obtain self-diffusion coefficients. The self-diffusion coefficients of water  $D_w$ , and of Pluronic  $D_P$  were determined at three different temperatures. Table 2 reports the self-diffusion coefficients obtained for the water signal (water solution containing 20 wt% of  $D_2O$  was used for lock purpose) and for the NMR signals  $CH_2$  and  $CH_3$  of the Pluronic F127 polymer (20 wt). The NaCMC (4 wt%) NMR signals have very low intensities.

Starting with the water diffusion coefficients  $D_w^{obs}$  values are within experimental error equal in the sample with and without added NaCl. The increase in the rate of water diffusion is mainly due to the change in water viscosity with temperature. Introducing the solvent viscosity  $\eta_w^0$  reported in Table S3 (SI), the product  $D_w^0(T) \times \eta_w^0(T)$  (see Eq. S6) is reasonably constant: for the salt free sample F/C; the values, determined at 16, 30, and 46 °C, are  $1.0 \cdot 10^{-12} \text{ kg m s}^{-2}$ ,  $1.12 \cdot 10^{-12} \text{ kg m s}^{-2}$  and  $1.19 \cdot 10^{-12} \text{ kg m s}^{-2}$ , respectively. There is a definite increase with T as can be expected from dehydration of F127. Similar values are calculated for the sample with NaCl. These data also yield similar activation energies calculated from the Arrhenius plot of  $D_w^0$  values (see Figure S7 in SI)

We next turn to the diffusion data for F127. According to previous works [33,43] we note that the echo decays are non-mono-exponential, so they were analysed with a biexponential function (Eq. S5) (see an example in SI, Figures S8 a-b). This approach rests on the assumption that there are two fractions of F127 in slow exchange on the relevant NMR timescale (a few 100 ms), each with a well-defined diffusion coefficient. This is clearly an approximation as F127 is a polydisperse polymer (see also ref. [43] for a discussion on this issue).

**Table 2.** Observed NMR Self-Diffusion coefficients of water  $D_w^{obs}$  and of Pluronic F127  $D_P^{obs}$  for samples F/C and F/C\_4 at different temperatures.

Sample	T (°C)	$D_w^{obs}$	$D_P$		$D_P^{obs}$ (a)	$\rho_{fast}$ (b)
--------	--------	-------------	-------	--	-----------------	-------------------

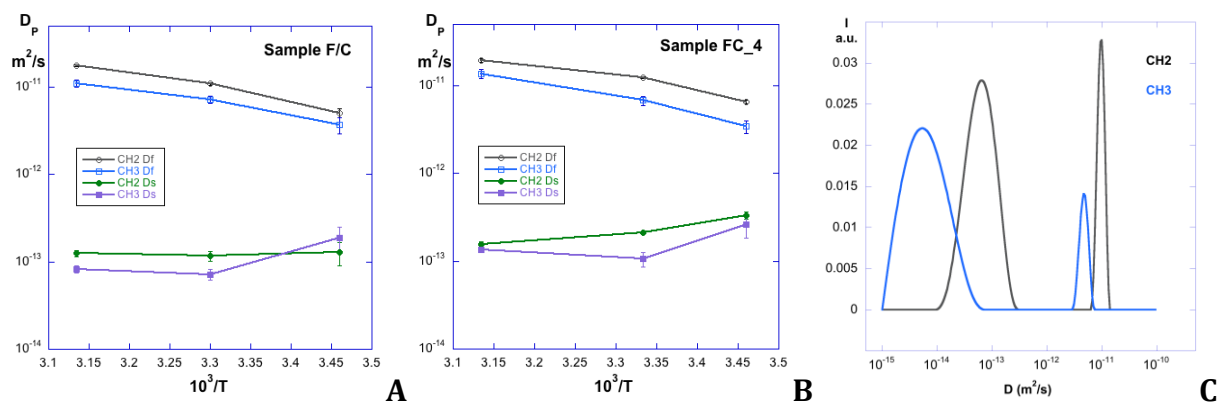
		$10^9 \text{ m}^2\text{s}^{-1}$			$\text{m}^2\text{s}^{-1}$	(eq. 5)
F/C no salt	16	$0.77 \pm 0.007$	$D_{\text{fast}}$	CH2	$5.06 \pm 0.6 \times 10^{-12}$	$0.304 \pm 0.043$
			$D_{\text{slow}}$		$1.29 \pm 0.38 \times 10^{-13}$	
			$D_{\text{fast}}$	CH3	$3.7 \pm 0.8 \times 10^{-12}$	$0.14 \pm 0.04$
			$D_{\text{slow}}$		$1.9 \pm 0.6 \times 10^{-13}$	
	30	$1.18 \pm 0.01$	$D_{\text{fast}}$	CH2	$1.09 \pm 0.04 \times 10^{-11}$	$0.211 \pm 0.005$
			$D_{\text{slow}}$		$1.17 \pm 0.15 \times 10^{-13}$	
			$D_{\text{fast}}$	CH3	$7.22 \pm 0.69 \times 10^{-12}$	$0.076 \pm 0.007$
			$D_{\text{slow}}$		$7.2 \pm 1 \times 10^{-14}$	
	46	$1.72 \pm 0.02$	$D_{\text{fast}}$	CH2	$1.77 \pm 0.05 \times 10^{-11}$	$0.175 \pm 0.003$
			$D_{\text{slow}}$		$1.25 \pm 0.1 \times 10^{-13}$	
			$D_{\text{fast}}$	CH3	$1.11 \pm 0.11 \times 10^{-11}$	$0.046 \pm 0.003$
			$D_{\text{slow}}$		$8.3 \pm 0.8 \times 10^{-14}$	
F/C_4 NaCl 0.07	16	$0.82 \pm 0.008$	$D_{\text{fast}}$	CH2	$6.56 \pm 0.26 \times 10^{-12}$	$0.29 \pm 0.01$
			$D_{\text{slow}}$		$3.35 \pm 0.3 \times 10^{-13}$	
			$D_{\text{fast}}$	CH3	$3.44 \pm 0.57 \times 10^{-12}$	$0.21 \pm 0.05$
			$D_{\text{slow}}$		$2.64 \pm 0.8 \times 10^{-13}$	
	27	$1.15 \pm 0.01$	$D_{\text{fast}}$	CH2	$1.23 \pm 0.03 \times 10^{-11}$	$0.200 \pm 0.003$
			$D_{\text{slow}}$		$2.10 \pm 0.09 \times 10^{-13}$	
			$D_{\text{fast}}$	CH3	$6.8 \pm 0.8 \times 10^{-12}$	$0.084 \pm 0.009$
			$D_{\text{slow}}$		$1.07 \pm 0.2 \times 10^{-13}$	
	46	$1.72 \pm 0.02$	$D_{\text{fast}}$	CH2	$1.94 \pm 0.08 \times 10^{-11}$	$0.173 \pm 0.003$
			$D_{\text{slow}}$		$1.57 \pm 0.11 \times 10^{-13}$	
			$D_{\text{fast}}$	CH3	$1.36 \pm 0.16 \times 10^{-11}$	$0.039 \pm 0.002$
			$D_{\text{slow}}$		$1.36 \pm 0.07 \times 10^{-13}$	

(a) the relatively high errors reported for  $D_{\text{slow}}$ , particularly for CH<sub>3</sub> signal is because the maximum  $G^2$  (Eq. 5) values only reduce the echo-intensities by around 10-30 % in all experiments

(b) from the exponential fitting of Eq. 5 (using Kaleidagraph), the  $p_{\text{slow}}$  fraction is calculated as  $p_{\text{slow}} = 1 - p_{\text{fast}}$ .

The weight factor of the slow component  $p_{\text{slow}}$  in the biexponential fits is around 0.8 - 0.95, except at  $T = 16 \text{ }^\circ\text{C}$ , when both samples are in the sol phase and show a higher content of the fast component  $p_{\text{fast}}$  around 0.3. If one were to take relaxation weighting into account,[33] the  $p_{\text{slow}}$  fraction would be even higher as the NMR relaxation rates in the aggregated Pluronic component is presumably faster than for the “free” component.

The two components can be identified as “aggregated” (giving rise to the slow diffusing component  $D_{\text{slow}}$ ) and “free” (giving rise to the fast component  $D_{\text{fast}}$ ) F127, and the quoted values of the diffusion coefficients should be regarded as averages of each fraction. To facilitate the further discussion, we present in Figure 5(A-B) the F127 diffusion data from Table 2.



**Figure 5.** NMR self-diffusion coefficients of the two components  $D_{p^{\text{fast}}}$  and  $D_{p^{\text{slow}}}$  of F127 as a function of  $T$  ( $10^3/T$ ), for both CH<sub>2</sub> and CH<sub>3</sub> groups, determined in sample (A) F/C and (B) F/C\_4. The error bars are obtained from the biexponential fitting. (C) Distribution of  $D_{\text{fast}}$  and  $D_{\text{slow}}$  for CH<sub>2</sub> and CH<sub>3</sub> NMR echo-decay at 30°C, for sample F/C in the gel phase. An in-house Matlab code, based on the work by Whittall and MacKay was used. [99]

The diffusion coefficients obtained from the CH<sub>3</sub> group of the PO differs from those of the CH<sub>2</sub> of the EO unit for both the slow and the fast components. Although the difference is not substantial, we judge it to be a real effect. The difference reflects the polydispersity of the F127 triblock polymer. For a true biexponential situation (e.g. a monodisperse micellar component and a monodisperse “free” polymer) the diffusion coefficient of the CH<sub>2</sub> and CH<sub>3</sub> groups must be the same. For a polydisperse situation (with slow exchange between aggregated and “free” Pluronic) the effect is caused by the different ratios of EO to PO in the two populations. Inverse Laplace transforms (ILT) of the echo decays provide a more general way of treating non-monoeponential echo decays (see ref. [43] for details). An ILT analysis for the F/C sample at 30°C is shown in Figure 5C, giving further support for the discussion above.

Turning to the diffusion of the fast component it increases, as expected, with temperature as reported in Figure 5A-5B (lines black and blue). The increase cannot be explained by the temperature dependence of the solvent viscosity (see viscosity data in Table S3 in SI for H<sub>2</sub>O/D<sub>2</sub>O = 80/20 mixture) alone but reflects the fractionation of the polydisperse Pluronic with temperature. This can clearly be seen in Figure 5A-5B where the  $D_{p^{fast}}$  values (reported on a log scale) do not show a linear dependence on  $1/T$  as expected for a single molecular moiety. As temperature increases the more hydrophilic components, with higher EO/PO ratios and lower molecular weights remain in solution, as previously demonstrated.[43] Here, F127 unimers diffuse in a lattice of Pluronic micelles, where the latter make up a volume fraction  $\Phi$  around 0.2.

As to the slow component diffusion coefficients, shown in Figure 5A-B (lines green and violet), they do not vary or decrease with temperature. Indeed,  $D_{p^{slow}}$  reflects the facts that the aggregates sit on a lattice, and diffuse very slowly, implying that the diffusion process is different for the aggregated F127 as compared to the free component. According to Mortensen,[35,36] for highly purified F127, the structure in the hard gel is BCC with a lattice constant of 280 Å for a 20 wt % solution. The distance  $l$  between the micelles is thus 140 Å. The diffusion process is a random walk between neighboring micelles. Such a process is described by  $D = \langle l^2 \rangle / (6\tau_R)$  where  $\tau_R$  is the residence time in the micelle. For  $D \approx 10^{-13} \text{ m}^2\text{s}^{-1}$  we obtain an average residence time of the Pluronic molecule in the micelle of 0.3 ms. This corresponds to an exit rate constant of  $3 \cdot 10^3 \text{ s}^{-1}$ . Since we have a distribution of the size of the hydrophobic PO unit in the Pluronic, we also get a distribution of lifetimes, which fact explains the different values for diffusion of the EO and PO units.

Finally, we note that the addition of NaCl to the F127/NaCMC sample affects the diffusion of the F127 molecules only slightly.

We end this section by discussing the dimension of the free (non-aggregated) F127. The self-diffusion coefficients are often used to evaluate hydrodynamic radius using the Stokes-Einstein equation corrected for obstruction effects due to the volume fraction of the dispersed phase, according to Eq. S7.

**Table 3.**  $D_p^0(fast)$  (eq. 7) of CH<sub>2</sub> and CH<sub>3</sub> groups,  $\eta$  and calculated radius  $R_P$  of free F127 moiety in samples F/C (no salt) and F/C\_4 (NaCl 0.07 mol/kg)  $D_p^0(fast)$

T (°C)	Group	$D_p^0(fast) \times 10^{11}$ m <sup>2</sup> /s	$\eta_w$ mPa s	$R_P$ nm
<b>F/C (no salt)</b>				
16	CH <sub>2</sub>	1.01	1.1789	17.7
	CH <sub>3</sub>	0.74		24.3
30	CH <sub>2</sub>	2.18	0.8660	11.8
	CH <sub>3</sub>	1.44		17.7
46	CH <sub>2</sub>	3.54	0.6293	10.5
	CH <sub>3</sub>	2.22		16.7
<b>F/C_4 + NaCl</b>				
16	CH <sub>2</sub>	1.31	1.1789	13.7
	CH <sub>3</sub>	0.69		26.1
27	CH <sub>2</sub>	2.46	0.9230	9.7
	CH <sub>3</sub>	1.36		17.5
46	CH <sub>2</sub>	4.12	0.6293	9.6
	CH <sub>3</sub>	3.88		13.6

Due to the trends observed in Table 2 and Figure 5A-5B, only the fast component  $D_p^{fast}$  can be considered to evaluate the hydrodynamic radius  $R_P$  of F127 unimers. Table 3 reports the  $D_p^0(fast)$  corrected for obstruction according to Eq. S7 ( $k\Phi = 0.5$ ), the solvent viscosity  $\eta_w$  (see Table S3), and the obtained  $R_P$  values. Despite the approximations made, these  $R_P$  values are likely to be reliable and representative of polymer unimers' dimensions that move in a water medium where a high MW polyelectrolyte such as NaCMC is dissolved and modifies not only the rheological behaviour but also the intermolecular interactions that influence the bulk

viscosity. As expected on the basis of previous findings,  $R_P$  values obtained for  $\text{CH}_2$  groups are always lower than those calculated for  $\text{CH}_3$  groups, and likely more reliable due to the much higher intensity of the  $\text{CH}_2$  NMR signal. Some  $R_P$  values are higher than the average hydrodynamic radius around 10 nm, reported for a F127 1 wt% aqueous solution in the range of temperature 30-60 °C.[44]

Finally, we may just note that with increasing temperature the dimensions of the diffusing species slightly decrease according to the increase of the self-diffusion coefficients  $D^{\circ}_{p^{fast}}$ .

#### 4. Concluding Remarks

In summary, the main findings of this study are related to the performance of a polymers mixture made by the thermosensitive Pluronic F127 (MW  $\approx$  12.5 kDa) and the polyelectrolyte NaCMC (MW  $\approx$  90 kDa), prepared in a weight ratio F/C = 20/4, in the presence of added salts at low concentration. Both NaCMC and salts affect transition temperatures: particularly NaCMC plays a crowding role that decreases the temperatures of the F127 cmt and of the sol-gel transition, as expected for an effective increase of F127 concentration; salt addition leads to a decrease of NaCMC crowding effect as a result of electrostatic interactions. Specific ion effects were particularly highlighted by the trend of complex viscosity whose trend resembles that of the elastic modulus. The analysis of rheological data highlighted two important features: i) complex viscosity and elastic modulus  $G'$  are strictly related and strikingly halve with increasing salt concentration from 0.05 to 0.07 mol/kg; ii) the addition of NaCl at the specific concentration of 0.07 mol/kg, induced a sol-gel transition temperature around 35 °C. NMR self-diffusion data suggested that NaCl addition did not significantly modify the Pluronic micellar assembly, likely due to the relatively low concentrations of the salts, and to the complex interactions addressed not only by crowding but also by a rebalancing between dispersive and electrostatic forces. Taken together, the findings of this study indicate that a mixture of Pluronic



F127/NaCMC (20/4 weight ratio), in the presence of NaCl at 0.07 mol/kg, is a suitable formulation platform for developing a potential thermosensitive drug delivery system, being the increase of the sol-(hard gel) transition temperature the main effect of the salt.

### **Author Contribution**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. All authors contributed equally.

### **Acknowledgements**

Financial supports from, FIR 2020, Fondazione di Sardegna (FdS, F72F20000230007), and Regione Autonoma della Sardegna (RASSR79857) are gratefully acknowledged. C.C. thanks MIUR (PON-AIM Azione I.2, DD 407- 27.02.2018, AIM1890410-2) for funding. O.S. thanks the Division of Physical Chemistry, Lund University, for financial support. B.L. acknowledges the Coimbra Chemistry Centre (CQC) FCT through the Project UID/QUI/00313/2020.

### **References**

- [1] B. Kronberg, K. Holmberg, B. Lindman, *Surface Chemistry of Surfactants and Polymers*, John Wiley & Sons, Ltd, Chichester, UK, 2014. doi:10.1002/9781118695968.
- [2] M. Malmsten, *Biopolymers at Interfaces*, CRC Press, 2003. doi:10.1201/9780824747343.
- [3] P. Alexandridis, Poly(ethylene oxide)/poly(propylene oxide) block copolymer surfactants, *Curr. Opin. Colloid Interface Sci.* 2 (1997) 478–489. doi:10.1016/S1359-0294(97)80095-7.
- [4] P. Alexandridis, B. Lindman, *Amphiphilic Block Copolymers*, Elsevier, 2000. doi:10.1016/B978-0-444-82441-7.X5000-2.
- [5] B. Lindman, G. Karlström, Nonionic polymers and surfactants: Temperature anomalies revisited, *Comptes Rendus Chim.* 12 (2009) 121–128. doi:10.1016/j.crci.2008.06.017.

- [6] A. Arnaiz, M. Guembe-García, E. Delgado-Pinar, A.J.M. Valente, S. Ibeas, J.M. García, S. Vallejos, The role of polymeric chains as a protective environment for improving the stability and efficiency of fluorogenic peptide substrates, *Sci. Rep.* 12 (2022) 8818. doi:10.1038/s41598-022-12848-4.
- [7] M.J. Vicent, F. Greco, R.I. Nicholson, A. Paul, P.C. Griffiths, R. Duncan, Polymer Therapeutics Designed for a Combination Therapy of Hormone-Dependent Cancer, *Angew. Chemie.* 117 (2005) 4129–4134. doi:10.1002/ange.200462960.
- [8] B. Lindman, B. Medronho, L. Alves, C. Costa, H. Edlund, M. Norgren, The relevance of structural features of cellulose and its interactions to dissolution, regeneration, gelation and plasticization phenomena, *Phys. Chem. Chem. Phys.* 19 (2017) 23704–23718. doi:10.1039/c7cp02409f.
- [9] B. Lindman, B. Medronho, L. Alves, M. Norgren, L. Nordenskiöld, Hydrophobic interactions control the self-assembly of DNA and cellulose, *Q. Rev. Biophys.* 54 (2021) e3. doi:10.1017/S0033583521000019.
- [10] E.H. Debutts, J.A. Hudy, J.H. Elliott, Rheology of Sodium Carboxymethylcellulose Solutions, *Ind. Eng. Chem.* 49 (1957) 94–98. doi:10.1021/ie50565a034.
- [11] W.-M. Kulicke, U. Reinhardt, G.G. Fuller, O. Arendt, Characterization of the flow properties of sodium carboxymethylcellulose via mechanical and optical techniques, *Rheol. Acta.* 38 (1999) 26–33. doi:10.1007/s003970050153.
- [12] C.G. Lopez, R.H. Colby, P. Graham, J.T. Cabral, Viscosity and Scaling of Semiflexible Polyelectrolyte NaCMC in Aqueous Salt Solutions, *Macromolecules.* 50 (2017) 332–338. doi:10.1021/acs.macromol.6b02261.
- [13] C.G. Lopez, R.H. Colby, J.T. Cabral, Electrostatic and Hydrophobic Interactions in NaCMC Aqueous Solutions: Effect of Degree of Substitution, *Macromolecules.* 51 (2018) 3165–3175. doi:10.1021/acs.macromol.8b00178.

- [14] A. Gandhi, A. Paul, S.O. Sen, K.K. Sen, Studies on thermoresponsive polymers: Phase behaviour, drug delivery and biomedical applications, *Asian J. Pharm. Sci.* 10 (2015) 99–107. doi:10.1016/j.ajps.2014.08.010.
- [15] F.D. Jochum, P. Theato, Temperature- and light-responsive smart polymer materials, *Chem. Soc. Rev.* 42 (2013) 7468–7483. doi:10.1039/c2cs35191a.
- [16] D. Schmaljohann, Thermo- and pH-responsive polymers in drug delivery, *Adv. Drug Deliv. Rev.* 58 (2006) 1655–1670. doi:10.1016/j.addr.2006.09.020.
- [17] P.D. Thornton, R.J. Mart, R. V. Ulijn, Enzyme-responsive polymer hydrogel particles for controlled release, *Adv. Mater.* 19 (2007) 1252–1256. doi:10.1002/adma.200601784.
- [18] Q. Zhang, C. Weber, U.S. Schubert, R. Hoogenboom, Thermoresponsive polymers with lower critical solution temperature: from fundamental aspects and measuring techniques to recommended turbidimetry conditions, *Mater. Horizons.* 4 (2017) 109–116. doi:10.1039/C7MH00016B.
- [19] E. Jin, Z. Zhang, H. Lian, X. Chen, C. Xiao, X. Zhuang, X. Chen, Injectable electroactive hydrogels based on Pluronic® F127 and tetraaniline copolymer, *Eur. Polym. J.* 88 (2017) 67–74. doi:10.1016/j.eurpolymj.2017.01.013.
- [20] Y.J. Kim, Y.T. Matsunaga, Thermo-responsive polymers and their application as smart biomaterials, *J. Mater. Chem. B.* 5 (2017) 4307–4321. doi:10.1039/c7tb00157f.
- [21] S. Andersson, M.J. Lidin, K. Larsson, Structure of the cubosome – a closed lipid bilayer aggregate, *Zeitschrift Für Krist. - Cryst. Mater.* 210 (1995) 315–318. doi:10.1524/zkri.1995.210.5.315.
- [22] M.A. Ward, T.K. Georgiou, Thermoresponsive polymers for biomedical applications, *Polymers (Basel).* 3 (2011) 1215–1242. doi:10.3390/polym3031215.
- [23] P. Hiwale, S. Lampis, G. Conti, C. Caddeo, S. Murgia, A.M. Fadda, M. Monduzzi, In Vitro Release of Lysozyme from Gelatin Microspheres: Effect of Cross-linking Agents and

- Thermoreversible Gel as Suspending Medium, *Biomacromolecules*. 12 (2011) 3186–3193. doi:10.1021/bm200679w.
- [24] M. Santin, C. Morris, G. Standen, L. Nicolais, L. Ambrosio, A new class of bioactive and biodegradable soybean-based bone fillers, *Biomacromolecules*. 8 (2007) 2706–2711.
- [25] L. Mayol, F. Quaglia, A. Borzacchiello, L. Ambrosio, M.I.L. Rotonda, A novel poloxamers/hyaluronic acid in situ forming hydrogel for drug delivery: Rheological, mucoadhesive and in vitro release properties, *Eur. J. Pharm. Biopharm.* 70 (2008) 199–206. doi:10.1016/j.ejpb.2008.04.025.
- [26] L. Mayol, M. Biondi, F. Quaglia, S. Fusco, A. Borzacchiello, L. Ambrosio, M.I. La Rotonda, Injectable thermally responsive mucoadhesive gel for sustained protein delivery, *Biomacromolecules*. 12 (2011) 28–33.
- [27] A. Borzacchiello, L. Russo, B.M. Malle, K. Schwach-Abdellaoui, L. Ambrosio, Hyaluronic acid based hydrogels for regenerative medicine applications, *Biomed Res. Int.* 2015 (2015). doi:10.1155/2015/871218.
- [28] G. Rajoria, A. Gupta, In-Situ Gelling System: A Novel Approach for Ocular Drug Delivery, *J. PharmTech Res.* 2 (2012) 24–53.
- [29] M.H.M. Nascimento, M.K.K.D. Franco, F. Yokaichyia, E. de Paula, C.B. Lombello, D.R. de Araujo, Hyaluronic acid in Pluronic F-127/F-108 hydrogels for postoperative pain in arthroplasties: Influence on physico-chemical properties and structural requirements for sustained drug-release, *Int. J. Biol. Macromol.* 111 (2018) 1245–1254. doi:10.1016/j.ijbiomac.2018.01.064.
- [30] G. Wanka, H. Hoffmann, W. Ulbricht, Phase Diagrams and Aggregation Behavior of Poly(oxyethylene)-Poly(oxypropylene)-Poly(oxyethylene) Triblock Copolymers in Aqueous Solutions, *Macromolecules*. 27 (1994) 4145–4159. doi:10.1021/ma00093a016.

- [31] K. Mortensen, Structural studies of aqueous solutions of PEO - PPO - PEO triblock copolymers, their micellar aggregates and mesophases; a small-angle neutron scattering study, *J. Phys. Condens. Matter.* 8 (1996) A103–A124. doi:10.1088/0953-8984/8/25A/008.
- [32] S. Liu, L. Li, Multiple Phase Transition and Scaling Law for Poly(ethylene oxide)–Poly(propylene oxide)–Poly(ethylene oxide) Triblock Copolymer in Aqueous Solution, *ACS Appl. Mater. Interfaces.* 7 (2015) 2688–2697. doi:10.1021/am507749w.
- [33] M. Shaikhullina, A. Khaliullina, R. Gimatdinov, A. Butakov, V. Chernov, A. Filippov, NMR relaxation and self-diffusion in aqueous micellar gels of pluronic F-127, *J. Mol. Liq.* 306 (2020) 112898. doi:10.1016/j.molliq.2020.112898.
- [34] C. Chaibundit, N.M.P.S.P.S. Ricardo, F. de M.L.L. Costa, S.G. Yeates, C. Booth, Micellization and Gelation of Mixed Copolymers P123 and F127 in Aqueous Solution, *Langmuir.* 23 (2007) 9229–9236. doi:10.1021/la701157j.
- [35] K. Mortensen, Y. Talmon, Cryo-TEM and SANS Microstructural Study of Pluronic Polymer Solutions, *Macromolecules.* 28 (1995) 8829–8834. doi:10.1021/ma00130a016.
- [36] K. Mortensen, W. Batsberg, S. Hvidt, Effects of PEO–PPO Diblock Impurities on the Cubic Structure of Aqueous PEO–PPO–PEO Pluronic Micelles: fcc and bcc Ordered Structures in F127, *Macromolecules.* 41 (2008) 1720–1727. doi:10.1021/ma702269c.
- [37] J.-P. Habas, E. Pavie, A. Lapp, J. Peyrelasse, Non-linear viscoelastic properties of ordered phases of a poly(ethylene oxide)-poly(propylene oxide) triblock copolymer, *Rheol. Acta.* 47 (2008) 765–776. doi:10.1007/s00397-008-0293-0.
- [38] M. Jalaal, G. Cottrell, N. Balmforth, B. Stoeber, On the rheology of Pluronic F127 aqueous solutions, *J. Rheol. (N. Y. N. Y).* 61 (2017) 139–146. doi:10.1122/1.4971992.
- [39] K. Suman, S. Sourav, Y.M. Joshi, Rheological signatures of gel–glass transition and a

revised phase diagram of an aqueous triblock copolymer solution of Pluronic F127, *Phys. Fluids*. 33 (2021) 073610. doi:10.1063/5.0057090.

- [40] M. Malmsten, B. Lindman, Effects of homopolymers on the gel formation in aqueous block copolymer solutions, *Macromolecules*. 26 (1993) 1282–1286. doi:10.1021/ma00058a014.
- [41] P. Holmqvist, P. Alexandridis, B. Lindman, Modification of the microstructure in poloxamer Block copolymer-water- "oil" systems by varying the "oil" type, *Macromolecules*. 30 (1997) 6788–6797.
- [42] J. Jansson, K. Schillén, M. Nilsson, O. Söderman, G. Fritz, A. Bergmann, O. Glatter, Small-angle X-ray scattering, light scattering, and NMR study of PEO-PPO-PEO triblock copolymer/cationic surfactant complexes in aqueous solution, *J. Phys. Chem. B*. 109 (2005) 7073–7083. doi:10.1021/jp0468354.
- [43] M. Nilsson, B. Håkansson, O. Söderman, D. Topgaard, Influence of Polydispersity on the Micellization of Triblock Copolymers Investigated by Pulsed Field Gradient Nuclear Magnetic Resonance, *Macromolecules*. 40 (2007) 8250–8258. doi:10.1021/ma071302p.
- [44] M. Malmsten, B. Lindman, Self-assembly in aqueous block copolymer solutions, *Macromolecules*. 25 (1992) 5440–5445. doi:10.1021/ma00046a049.
- [45] M. Malmsten, B. Lindman, Water self-diffusion in aqueous block copolymer solutions, *Macromolecules*. 25 (1992) 5446–5450. doi:10.1021/ma00046a050.
- [46] N. Pandit, Loss of gelation ability of Pluronic® F127 in the presence of some salts, *Int. J. Pharm.* 145 (1996) 129–136. doi:10.1016/S0378-5173(96)04748-5.
- [47] N. Pandit, T. Trygstad, S. Croy, M. Bohorquez, C. Koch, Effect of salts on the micellization, clouding, and solubilization behavior of pluronic F127 solutions, *J. Colloid Interface Sci.* 222 (2000) 213–220. doi:10.1006/jcis.1999.6628.

- [48] P. Liu, M. Zhai, J. Li, J. Peng, J. Wu, Radiation preparation and swelling behavior of sodium carboxymethyl cellulose hydrogels, *Radiat. Phys. Chem.* 63 (2002) 525–528. doi:10.1016/S0969-806X(01)00649-1.
- [49] M.A. Cancela, E. Álvarez, R. Maceiras, Effects of temperature and concentration on carboxymethylcellulose with sucrose rheology, *J. Food Eng.* 71 (2005) 419–424. doi:10.1016/j.jfoodeng.2004.10.043.
- [50] X.H. Yang, W.L. Zhu, Viscosity properties of sodium carboxymethylcellulose solutions, *Cellulose.* 14 (2007) 409–417. doi:10.1007/s10570-007-9137-9.
- [51] F. Cuomo, F. Lopez, M. Piludu, M.G. Miguel, B. Lindman, A. Ceglie, Release of small hydrophilic molecules from polyelectrolyte capsules: Effect of the wall thickness, *J. Colloid Interface Sci.* 447 (2015) 211–216. doi:10.1016/j.jcis.2014.10.060.
- [52] B. Medronho, B. Lindman, Brief overview on cellulose dissolution/regeneration interactions and mechanisms, *Adv. Colloid Interface Sci.* 222 (2015) 502–508. doi:10.1016/j.cis.2014.05.004.
- [53] P. Singh, B. Medronho, L. Alves, G.J. da Silva, M.G. Miguel, B. Lindman, Development of carboxymethyl cellulose-chitosan hybrid micro- and macroparticles for encapsulation of probiotic bacteria, *Carbohydr. Polym.* 175 (2017) 87–95. doi:10.1016/j.carbpol.2017.06.119.
- [54] J. Yashiro, R. Hagino, S. Sato, T. Norisuye, Chain stiffness and excluded-volume effects in polyelectrolyte solutions: Characterization of sodium poly(2-acrylamido-2-methylpropanesulfonate) in aqueous sodium chloride, *Polym. J.* 38 (2006) 57–63. doi:10.1295/polymj.38.57.
- [55] R. Hagino, J. Yashiro, M. Sakata, T. Norisuye, Electrostatic contributions to chain stiffness and excluded-volume effects in sodium poly(2-acrylamido-2-methylpropanesulfonate) solutions, *Polym. J.* 38 (2006) 861–867.

doi:10.1295/polymj.PJ2006013.

- [56] F. Hofmeister, Zur Lehre von der Wirkung der Salze, Arch. Für Exp. Pathol. Und Pharmakologie. 24 (1888) 247–260. doi:10.1007/BF01918191.
- [57] W. Kunz, J. Henle, B.W. Ninham, “Zur Lehre von der Wirkung der Salze” (about the science of the effect of salts): Franz Hofmeister’s historical papers, Curr. Opin. Colloid Interface Sci. 9 (2004) 19–37. doi:10.1016/j.cocis.2004.05.005.
- [58] Y. Zhang, P. Cremer, Interactions between macromolecules and ions: the Hofmeister series, Curr. Opin. Chem. Biol. 10 (2006) 658–663. doi:10.1016/j.cbpa.2006.09.020.
- [59] J. Lyklema, Simple Hofmeister series, Chem. Phys. Lett. 467 (2009) 217–222. doi:10.1016/j.cplett.2008.11.013.
- [60] A. Salis, M. Cristina Pinna, D. Bilaničová, M. Monduzzi, P. Lo Nostro, B.W. Ninham, Specific Anion Effects on Glass Electrode pH Measurements of Buffer Solutions: Bulk and Surface Phenomena, J. Phys. Chem. B. 110 (2006) 2949–2956. doi:10.1021/jp0546296.
- [61] A. Salis, D. Bilaničová, B.W. Ninham, M. Monduzzi, Hofmeister Effects in Enzymatic Activity: Weak and Strong Electrolyte Influences on the Activity of *Candida rugosa* Lipase, J. Phys. Chem. B. 111 (2007) 1149–1156. doi:10.1021/jp066346z.
- [62] P. Lo Nostro, B.W. Ninham, Hofmeister Phenomena: An Update on Ion Specificity in Biology, Chem. Rev. 112 (2012) 2286–2322. doi:10.1021/cr200271j.
- [63] A. Salis, B.W. Ninham, Models and mechanisms of Hofmeister effects in electrolyte solutions, and colloid and protein systems revisited, Chem. Soc. Rev. 43 (2014) 7358–7377. doi:10.1039/c4cs00144c.
- [64] C. Carucci, A. Salis, E. Magner, Electrolyte effects on enzyme electrochemistry, Curr. Opin. Electrochem. 5 (2017) 158–164. doi:10.1016/j.coelec.2017.08.011.
- [65] C. Carucci, A. Salis, E. Magner, Specific Ion Effects on the Mediated Oxidation of NADH,



- ChemElectroChem. 4 (2017) 3075–3080. doi:10.1002/celc.201700672.
- [66] Y. Marcus, Surface Tension of Aqueous Electrolytes and Ions, *J. Chem. Eng. Data.* 55 (2010) 3641–3644. doi:10.1021/je1002175.
- [67] Y. Marcus, Individual ionic surface tension increments in aqueous solutions, *Langmuir.* 29 (2013) 2881–2888. doi:10.1021/la3041659.
- [68] Y. Marcus, Specific ion effects on the surface tension and surface potential of aqueous electrolytes, *Curr. Opin. Colloid Interface Sci.* 23 (2016) 94–99. doi:10.1016/j.cocis.2016.06.016.
- [69] Y. Marcus, A relationship between the effect of uni-univalent electrolytes on the structure of water and on its volatility, *J. Chem. Phys.* 148 (2018). doi:10.1063/1.5009311.
- [70] R. Breslow, T. Guo, Surface tension measurements show that chaotropic salting-in denaturants are not just water-structure breakers., *Proc. Natl. Acad. Sci.* 87 (1990) 167–169. doi:10.1073/pnas.87.1.167.
- [71] Y. Zhang, P.S. Cremer, Chemistry of Hofmeister anions and osmolytes, *Annu. Rev. Phys. Chem.* 61 (2010) 63–83. doi:10.1146/annurev.physchem.59.032607.093635.
- [72] Y. Zhang, S. Furyk, D.E. Bergbreiter, P.S. Cremer, Specific ion effects on the water solubility of macromolecules: PNIPAM and the Hofmeister series, *J. Am. Chem. Soc.* 127 (2005) 14505–14510. doi:10.1021/ja0546424.
- [73] E. Thormann, On understanding of the Hofmeister effect: how addition of salt alters the stability of temperature responsive polymers in aqueous solutions, *RSC Adv.* 2 (2012) 8297. doi:10.1039/c2ra20164j.
- [74] L.M. Pegram, M.T. Record, Hofmeister salt effects on surface tension arise from partitioning of anions and cations between bulk water and the air-water interface, *J. Phys. Chem. B.* 111 (2007) 5411–5417. doi:10.1021/jp070245z.

- [75] L.M. Pegram, M.T. Record, Thermodynamic origin of hofmeister ion effects, *J. Phys. Chem. B.* 112 (2008) 9428–9436. doi:10.1021/jp800816a.
- [76] L.M. Pegram, M.T. Record, Quantifying accumulation or exclusion of H<sup>+</sup>, HO<sup>-</sup>, and Hofmeister salt ions near interfaces, *Chem. Phys. Lett.* 467 (2008) 1–8. doi:10.1016/j.cplett.2008.10.090.
- [77] J.C. Lutter, T. Wu, Y. Zhang, Hydration of Cations: A Key to Understanding of Specific Cation Effects on Aggregation Behaviors of PEO-PPO-PEO Triblock Copolymers, *J. Phys. Chem. B.* 117 (2013) 10132–10141. doi:10.1021/jp405709x.
- [78] M. Boström, D.F. Parsons, A. Salis, B.W. Ninham, M. Monduzzi, Possible Origin of the Inverse and Direct Hofmeister Series for Lysozyme at Low and High Salt Concentrations, *Langmuir.* 27 (2011) 9504–9511. doi:10.1021/la202023r.
- [79] A. Salis, F. Cugia, D.F. Parsons, B.W. Ninham, M. Monduzzi, Hofmeister series reversal for lysozyme by change in pH and salt concentration: insights from electrophoretic mobility measurements, *Phys. Chem. Chem. Phys.* 14 (2012) 4343. doi:10.1039/c2cp40150a.
- [80] M. Bohorquez, C. Koch, T. Trygstad, N. Pandit, A Study of the Temperature-Dependent Micellization of Pluronic F127, *J. Colloid Interface Sci.* 216 (1999) 34–40. doi:10.1006/jcis.1999.6273.
- [81] C.W. Macosko, *Rheology Principles, Measurements and Applications*, Wiley-vch New York, 1994.
- [82] P. Stilbs, Fourier transform pulsed-gradient spin-echo studies of molecular diffusion, *Prog. Nucl. Magn. Reson. Spectrosc.* 19 (1987) 1–45. doi:10.1016/0079-6565(87)80007-9.
- [83] P. Stilbs, K. Paulsen, Global least-squares analysis of large, correlated spectral data sets and application to chemical kinetics and time-resolved fluorescence, *Rev. Sci. Instrum.*

- 67 (1996) 4380–4386. doi:10.1063/1.1147539.
- [84] W.S. Price, Pulsed-field gradient nuclear magnetic resonance as a tool for studying translational diffusion: Part II. Experimental aspects, *Concepts Magn. Reson.* 10 (1998) 197–237. doi:10.1002/(SICI)1099-0534(1998)10:4<197::AID-CMR1>3.0.CO;2-S.
- [85] G.M. Bell, Self-diffusion of ions in the electric fields of spherical particles, *Trans. Faraday Soc.* 60 (1964) 1752. doi:10.1039/tf9646001752.
- [86] B. Faucompré, B. Lindman, Self-association of zwitterionic and nonionic surfactants. NMR self-diffusion studies, *J. Phys. Chem.* 91 (1987) 383–389. doi:10.1021/j100286a027.
- [87] K. Zhang, M. Jonstroemer, B. Lindman, Interaction between Nonionic Micelles and a Nonionic Polymer Studied by Fourier Transform NMR Self-Diffusion, *J. Phys. Chem.* 98 (1994) 2459–2463. doi:10.1021/j100060a038.
- [88] P. Holmqvist, P. Alexandridis, B. Lindman, Phase behavior and structure of ternary amphiphilic block copolymer-alkanol-water systems: Comparison of poly(ethylene oxide)/poly(propylene oxide) to poly(ethylene oxide)/poly(tetrahydrofuran) copolymers, *Langmuir.* 13 (1997) 2471–2477.
- [89] K. Mortensen, Structural properties of self-assembled polymeric micelles, *Curr. Opin. Colloid Interface Sci.* 3 (1998) 12–19. doi:10.1016/S1359-0294(98)80036-8.
- [90] P. Alexandridis, J.F. Holzwarth, Differential Scanning Calorimetry Investigation of the Effect of Salts on Aqueous Solution Properties of an Amphiphilic Block Copolymer (Ploxamer), *Langmuir.* 13 (1997) 6074–6082. doi:10.1021/la9703712.
- [91] L.E. Nita, A. Chiriac, M. Bercea, B.A. Wolf, Synergistic behavior of poly(aspartic acid) and Pluronic F127 in aqueous solution as studied by viscometry and dynamic light scattering, *Colloids Surfaces B Biointerfaces.* 103 (2013) 544–549. doi:10.1016/j.colsurfb.2012.10.054.

- [92] L.E. Nita, A.P. Chiriac, M. Bercea, Effect of pH and temperature upon self-assembling process between poly(aspartic acid) and Pluronic F127, *Colloids Surfaces B Biointerfaces*. 119 (2014) 47–54. doi:10.1016/j.colsurfb.2014.04.023.
- [93] J. Jiang, C. Li, J. Lombardi, R.H. Colby, B. Rigas, M.H. Rafailovich, J.C. Sokolov, The effect of physiologically relevant additives on the rheological properties of concentrated Pluronic copolymer gels, *Polymer (Guildf)*. 49 (2008) 3561–3567. doi:10.1016/j.polymer.2008.05.038.
- [94] B.A. Deyerle, Y. Zhang, Effects of Hofmeister Anions on the Aggregation Behavior of PEO–PPO–PEO Triblock Copolymers, *Langmuir*. 27 (2011) 9203–9210. doi:10.1021/la201463g.
- [95] X. Chen, T. Yang, S. Kataoka, P.S. Cremer, Specific ion effects on interfacial water structure near macromolecules, *J. Am. Chem. Soc.* 129 (2007) 12272–12279. doi:10.1021/ja073869r.
- [96] S.C. Barros, M.M. Silva, Seeking the lowest phase transition temperature in a cellulosic system for textile applications, *Cellulose*. 25 (2018) 3163–3178. doi:10.1007/s10570-018-1763-x.
- [97] I. Noda, T. Tsuge, M. Nagasawa, The intrinsic viscosity of polyelectrolytes, *J. Phys. Chem.* 74 (1970) 710–719. doi:10.1021/j100699a005.
- [98] Y. Zhang, S. Furyk, L.B. Sagle, Y. Cho, D.E. Bergbreiter, P.S. Cremer, Effects of Hofmeister Anions on the LCST of PNIPAM as a Function of Molecular Weight, *J. Phys. Chem. C*. 111 (2007) 8916–8924. doi:10.1021/jp0690603.
- [99] K.P. Whittall, A.L. MacKay, Quantitative interpretation of NMR relaxation data, *J. Magn. Reson.* 84 (1989) 134–152. doi:10.1016/0022-2364(89)90011-5.