Modifying the properties of thermogelling poloxamer 407 solutions through covalent modification and the use of polymer additives

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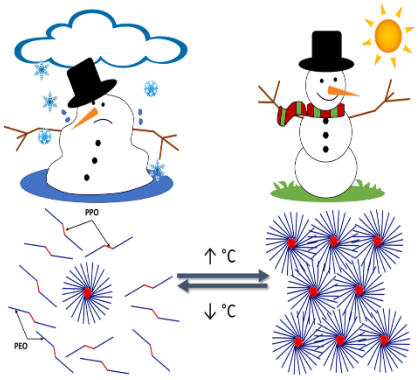
Abstract:

Thermoresponsive polymers that undergo sol-gel transition in a physiological temperature range have been drawing attention for biomedical applications as drug delivery systems. Poloxamer P407 is commonly used as thermogelling material and has already been approved by the Food and Drug Administration (FDA) in licenced medicines. However, its solutions have significant drawbacks which limit its performance, particularly in drug delivery systems. In order to improve these properties, the chemical structure of P407 has been modified to produce stronger gels while retaining the thermogelling properties by either conjugating P407 with other polymers or introducing inter-micelle linkers to the poloxamer ends. However, chemical modifications can have several undesirable side-effects because the change in the chemical structure makes the polymer a novel excipient, and additional safety risks are

possible, requiring expensive and time-consuming toxicity testing prior to regulatory approval. An alternative approach to covalent modification is modifying the P407 solution's formulations with additives including hydrophilic polymers (such as crosslinked polyacrylic acid, polyvinyl alcohol and polysaccharides) and nanoparticles. These additives have been used to enhance the P407 thermogel's properties. However, the majority of these studies fail to generate P407 gels with improved strength to the standard 20 % w/w solution used. This review investigates the approaches used to improve the properties of poloxamer 407 thermogelling materials, including the use of polymer additives and covalent modification. Several recommendations are made, based on efficacy and consideration of regulatory risk to guide the development of these materials toward use in real clinical applications.



Keywords: Bioprinting, self-assembly, drug delivery, hydrogels, pluronic

1. Introduction:

"Thermogels", "thermoresponsive materials", "Thermoreversible gels" or "thermogelling materials" are aqueous polymer solutions that exhibit a sol-gel transition above a critical temperature. Materials undergoing this transition in a physiological temperature range

(typically between 25 to 37 °C) have been drawing attention for biomedical applications as in-situ forming drug delivery systems, where the polymer solution may pass through an applicator before forming a gel upon contact with the body.[1] Drugs entrapped within the gel may then be retained at a site of action and elute from the gel with time. Thermogelling materials have also found use in bioprinting, tissue engineering, and diagnostics. Poloxamer 407, a poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol) triblock copolymer, is the most commonly used thermogelling material in published research. It is chemically stable,[2] has been used as an excipient in licenced medicines, and is inexpensive.[1] As such, it has been widely investigated for various biomedical applications, including drug delivery,[3] wound dressings,[4] and cell culture.[5] However, there are significant drawbacks for thermogelling poloxamer 407 solutions including insufficient gel strength for some applications, weak mucoadhesiveness, and rapid dissolution of the gels formed which has limited its performance, particularly in drug delivery systems.[6] To improve gel strength, researchers have investigated blending poloxamer with other hydrophilic polymers or nanoparticles as well as chemically modifying the structure of P407. However, whilst there are in-depth literature reviews on the subject of thermogelling materials [3,4,7–15], these articles lack a focus on how the material properties of P407 may be modified. In the following manuscript, we review the properties of poloxamer solutions and methods used to study them, followed by reported methods to modify poloxamer 407 solution properties using polymer blends and chemical modification. Throughout the manuscript we make recommendations regarding the future development of these materials for biomedical applications, considering the efficacy of the modifications weighed against any potential risks which the modifications may impart.

2. Poloxamer 407 as a thermogelling material

Pluronic® and Kolliphor® are BASF corporation trade names for poloxamer (Figure 1), also sold by Corda as Synperonic®.[11] Poloxamers are a group of poly(ethylene oxide)-poly(propylene glycol)-poly(ethylene oxide) (PEO–PPO–PEO) amphiphilic triblock copolymers of various block lengths.[16] These copolymers are non-ionic surfactants and can be synthesised with different molecular mass and PEO/PPO block ratios.[17] As the PEO (hydrophilic) sections increase in length relative to the PPO segment (hydrophobic), the hydrophile-lipophile balance (HLB) is expected to increase. The poloxamers' wide HLB range makes each one a different class of surfactant.[17] P184 has the formula $(EO)_{13}$–*b*–$(PO)_{30}$–*b*–$(EO)_{13,}$ an average molecular mass of 2900 Da. P408 has the formula $(EO)_{132}$–$(PO)_{50}$–$(EO)_{132}$ with an average molecular mass of 14,600 Da. Poloxamer 407 (P407), also known as Kolliphor® P407 or Pluronic® F127, has the structure $(EO)_{100}$–$(PO)_{65}$–$(EO)_{100}$, and an average molecular mass of 12600 Da.[17–20] P407 has a unique ability, in contrast to other poloxamer forms, to exhibit thermogelling behaviour at physiologically relevant temperatures. P407, when in a 20 % w/w solution, transforms from a relatively low viscosity solution below approximately 20 °C to a clear gel when warmed above this critical temperature. This makes it attractive for applications including injectable and topical pharmaceutical formulations, where the material may flow through an applicator or syringe before forming a gel upon contact with the body (35-37 °C).[22] These gels are also attractive for drug delivery because the material is chemically stable, even in the presence of acids, alkalis, and metal ions.[2] P407 is known to be well-tolerated by humans, and has already been approved by the FDA in licenced medicines, evidenced by listing in the inactive ingredients database.[23] P407's ability to form a gel at this low temperature has also made it attractive for bioprinting, where a mixture of cells and P407 may be printed into 3D structures from which tissue constructs are formed. These tissue constructs may be used in regenerative medicine applications [21].

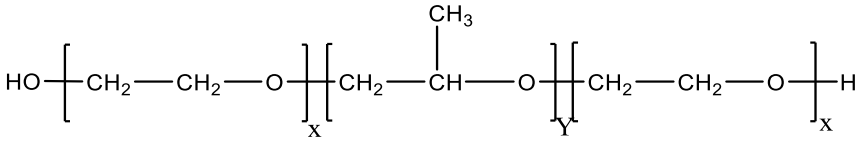


Figure 1: *General chemical structure for poloxamers $(PEO)_X$-$(PPO)_Y$-$(PEO)_X$. X and Y indicate the degree of polymerisation of each block.*

P407 has been widely investigated for use in pharmaceutical formulations for various routes of administration,[24] including opthalmic,[25] nasal,[26,27] vaginal,[28] rectal,[29] oral, transdermal,[30] and parenteral [31–34]. Currently, there are 19 medicines licenced by the FDA containing P407 as an inactive ingredient. One example is Otiprio®, a middle ear anti-inflammation medicine.[35] Another example is Omeprazole Delayed Release 20 mg which is used to reduce heartburn symptoms.[36] P407 is not listed in the FDA inactive ingredient for the parenteral route of administration. Listing in the inactive ingredients database significantly de-risks the use of P407 in future formulations as it evidences a history of use in humans. Thus, it is far more attractive to formulators than novel excipients, which would require separate clinical studies, potentially costing millions. Some examples of these listings are shown in Table 1, which includes the route of administration.

Table 1*: FDA approved formulations which include P407 as an inactive ingredient.*

Route	**Dosage Form**	**Maximum Potency[a]**
INTRATYMPANIC	SUSPENSION	15.1 % w/w
ORAL	SOLUTION	50 mg/5 mL
ORAL	CAPSULE, DELAYED ACTION	40 mg
PERIODONTAL	GEL	15.5 %

TOPICAL	LOTION	1 % w/w

[a] as described in the inactive ingredients database

2.1. Poloxamer rheology:

Rheology is used in pharmaceutical science as a way of characterisation and classification of liquid and semi-solid dosage forms, and to predict performance. The complex rheology of P407 means that careful consideration must be made to decide upon relevant testing methods either to characterise material properties or predict performance. Of relevance to both is the rheological properties of P407 in the solution and gel states, and when this transition occurs. The rheology of pure poloxamer 407 solutions is described herein to be used as a comparator in the remainder of the review.

The properties of the poloxamer gel at 37 °C can be explored by rheometry (Figure 2). Stress sweeps may be performed on 20 % w/w P407 to investigate the effect of increasing shear stress of gel properties (Figure 2A). The stress sweep identifies a linear viscoelastic region, at which the values of G' and G'' relate to gel strength, and the yield stress point, where G' and G'' cross and start rapidly decreasing (316.2 Pa). After this point the stress applied on the sample caused it to decrease in viscosity (shear thinning) because at higher shear rates there is a change in the material structure, allowing flow to occur more readily. A frequency sweep (Figure 2b) demonstrates the 20 % w/w P407 at 37 °C has a gel-like structure, as the G` was higher than the G`` and this was largely independent of frequency.[37] A temperature ramp for P407 20 % w/w solution is shown in Figure 2C. This data illustrates that at temperatures below 20.1 ± 0.3 °C, the values of storage modulus (G') are lower than the values for the loss modulus (G''), which indicates that the solution has liquid-like properties. Above this critical temperature, G' starts to become higher than G'', which indicates that the sample starts to exhibit more solid-like behaviour at that temperature. This point is considered the gelation temperature or the

transition temperature (Tgel). After this point, both the G' and G'' values increase rapidly until the temperature reaches 22.3 °C then starts to plateau. At 15 °C, the G' and G'' values were 0.04 ± 0.03 Pa and 0.29 ± 0.04 Pa, respectively. While at 35 °C, the values were 20 ± 1 kPa and 1.4 ± 0.2 kPa, respectively, indicating a dramatic increase in viscosity.

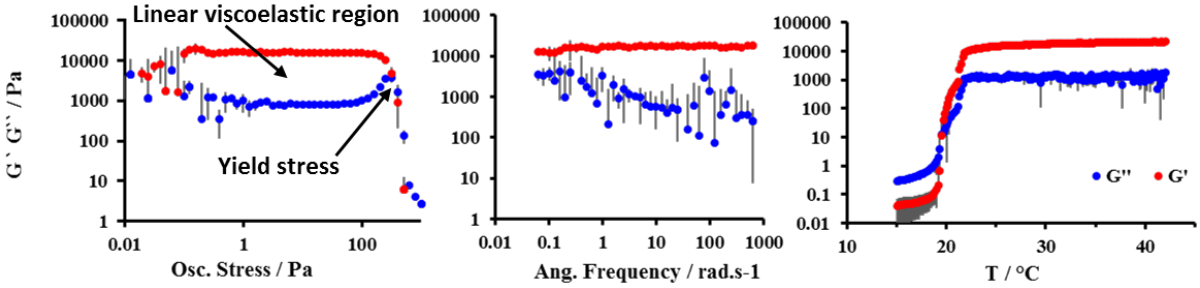


***Figure 2**: A) stress sweep for the P407 20 % w/w solution at 37 °C and 1 Hz frequency B) frequency sweep for the P407 20 % w/w solution at 37 °C and 1 Pa Stress),C) temperature ramps for a 20 % w/w P407 solution (unpublished data)* ● *G``* ● *G'*

2.2. The gelation mechanism of poloxamer 407:

The gelation mechanism of P407 has been widely investigated using ultrasonic velocity,[38] static and dynamic light-scattering,[38–40] cryogenic temperature transmission electron microscopy (cryo-TEM)[41], small-angle neutron scattering[39,41] and Fourier-transform infrared spectroscopy (FTIR).[42] It has been concluded that the gelation occurs via the formation of spherical micelles above a critical temperature.[2,43] At low temperature, and above the critical micelle concentration (CMC) of 1 mg/mL, P407 unimers equilibrate with its micelles in solution, with the micelles having a hydrodynamic radius of ca. 10 nm. The residence time of P407 in these micelles is extremely long (hours).[44] As the temperature increases, P407 unimers increasingly start to aggregate to form micelles because of the entropically-driven desolvation of PPO blocks,[45] accompanied by a reduction in the CMC.[44] This causes the equilibrium to shift towards a larger volume fraction of micelles (Ø> 0.53), which in turn leads to micelle packing and transition from liquid solution to gel as

shown in (Figure 3).[1,2,10,19] The size and aggregation number of the micelles are unchanged at the high concentrations and temperatures of the gel state,[18] which has been determined to have a face-centred cubic lattice by small-angle neutron scattering [46] and small-angle X-ray scattering. Cryo-TEM has also been used to visualise this lyotropic liquid crystalline phase [41].

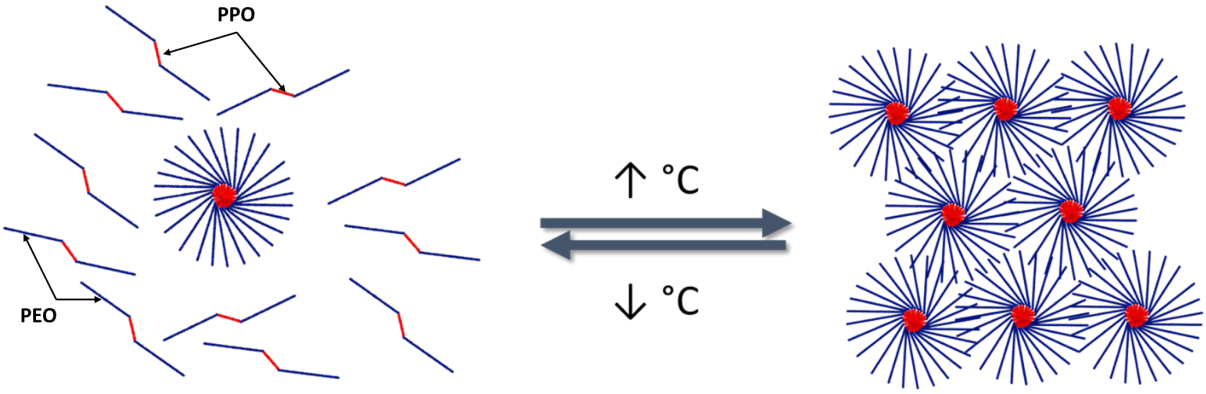


Figure 3: *an illustration of the P407 gelation when increasing temperature* [1]. *Heating above a critical temperature leads to an increase in the volume fraction of micelles, accompanied by packing to form a gel.*

2.3. Drawbacks of poloxamer 407 as a thermogelling material

Despite the attractive properties of P407 as a thermogelling material, there are several drawbacks for P407 hydrogels such as, weak mechanical strength due to P407's shear thinning character, rapid dissolution, and weak mucoadhesion, which has made it unfavourable for topical applications, or other areas where the gel may be affected by shear.[6,47,48] For instance, a 20 % w/w P407 formulation containing acyclovir was administered intravaginally to mice and after three hours only half of the acyclovir administered was detected, and none was found after six hours.[49] Thus, finding a way to improve retention is needed for prolonged topical drug delivery. The rheology of the gel is an important parameter for its performance as a dosage form and may be linked to factors such as retention.

2.4. Parameters used to describe Poloxamer 407 gels for drug delivery applications

To overcome the limitations of P407, various methods to modify the properties of P407 have been investigated. Two methods reported in the literature are the mixing of other hydrophilic polymers into P407 solutions, or by covalently modifying the structure of P407. Key parameters which may affect the performance of P407 as a dosage form are the sol-gel transition temperature (Tgel)[50], the rheology of the gel formed [51], dissolution rate, and the ability of the gel to adhere to mucosal membranes (mucoadhesion) [52]. Other parameters which may be of interest are rate of drug release from the gel, ability to retard or promote permeation through membranes, and ability to solubilise hydrophobic drugs, but these parameters are outside of the scope of this review.

Tgel is highly important for biomedical applications.[1] For instance, if Tgel was lower than room temperature there would be difficulty in the manufacture, handling and application of a medicine. On the other hand, if it was higher than body temperature (37 °C internally, and 34-35 °C on the surface) the solution would remain as a liquid after application.[53] In general, the Tgel of P407 solution decreases with increasing concentration, which may be due to the increased absolute number of micelles present in solution, resulting in the critical packing concentration being reached at lower temperatures.[54] Tgel may be determined using the inverted test-tube method, where the sample is placed in a vial or test-tube, then warmed in a water bath. The bath temperature which causes the sample to stop flowing when inverted would be considered the Tgel.[55] Another method of determining Tgel which has been reported is placing the sample in a vial containing a stirrer bar and heating the sample gradually while stirring at 50 rpm. Tgel would be the temperature that causes the stirrer bar to stop moving.[53] This method, though simple and cheap for any laboratory to perform has the limitation that the shear imposed on the gel will vary greatly dependent on rotation speed, magnetic follower size and shape, and the strength of the magnetic field. Finally, oscillatory rheometry has also been

used to measure Tgel and is the most accurate method. A temperature ramp may be performed on the sample (Figure 2C) where Tgel is the temperature where the storage modulus (G`) overcomes the loss modulus (G``). It is important to note, however, that there is an over-reliance on this cross-over point as a metric to determine gelation, and materials which appear to be a gel macroscopically may not have G` > G`` if the viscosity of the solution is sufficiently high to resist flow due to gravity.[51]

The strength of thermogels is crucial to their performance as a dosage form and may be measured by various techniques using instruments such as a texture analyser or rheometer to study response of the gels to applied forces. A rheometer is the most common tool used to study the properties of these gels, but the metric used as a measure of gel strength varies. Some authors report the dynamic viscosity at a specific temperature.[33] Alternatively, the value of G` obtained at given temperature may be used as a value of strength, where the higher the value of G`, the stronger the gel.[56] The G``/G` ratio, also known as tan(δ) or the loss tangent, is a third measure of "strength", where the smaller this ratio the more elastic the material would be.[57] In this context, elasticity refers to the ability to recover shape following deformation. Texture analysis may also be used to determine the mechanical response of gel formulations, using values such as "bloom strength". Without access to this equipment, some studies use bespoke experiments to give basic ranking of formulations. For instance, El-Khamil and El-Khatib used the weight necessary to force a disc 5 cm into a gel placed in graduated cylinder as a measure of gel strength.[53]

Dissolution in physical conditions is one of the major drawbacks of poloxamer formulations. It can be measured by adding to a gel sample an amount of buffer at 37 °C then removing the solvent and measuring the remaining gel volume or weight.[58] Dissolution has also been determined *in vivo* by measuring the attrition of a gel that has been injected into a rat.[59]

Mucoadhesion describes the force with which a dosage form binds to a mucus membrane to avoid the formulation leaking from the mucosal surface.[11] P407 solutions typically showed low mucoadhesion.[1,47,54,60,61] The mucoadhesion may be calculated as the force required to separate the gel from *ex vivo* mucosal tissue, often measured with a texture analyser.[62] The rheological method of Hassan and Gallo may also be used.[63] Mucin, a constituent of secretory mucus, is mixed with the gel and any synergistic interaction may be measured.[64] This rheological method has been criticized due to its lack of correlation with other techniques, and is often recommended to investigate mechanisms of interaction in these systems, rather than try to correlate this method with *in vivo* mucoadhesion. Texture analysis may also be used to measure synergistic interactions.[65] As an alternative, "flow-through" methods can be used to measure mucoadhesion. An *ex vivo* mucosal tissue would be placed on a channel then the material to be tested would be placed on top of it. After that, a physiologically relevant solution would be washed over the surface and then collected and analysed with an analytical technique such as High Performance Liquid Chromatography (HPLC) to quantify the concentration of the dissolved material.[52] Polymers may be tagged with fluorophores to detect their presence on the tissue by microscopy.[66,67] Overall, the techniques for measuring mucoadhesion are highly varied, lacking standardisation and validation, however the detachment method using texture analysis is the most common approach.[65,68]

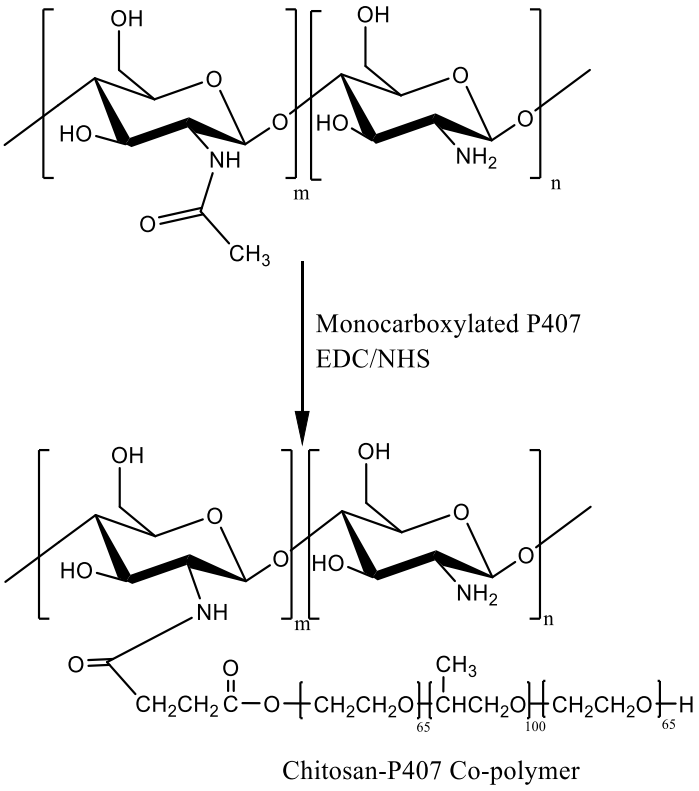
3. Chemical modification of poloxamer 407 to alter thermoresponsive gels

Poloxamer gels are very soft and easily fragmented and diluted in biological milieu causing disassembly of the micellar structure resulting in dissolution earlier than desired for prolonged action of a drug or protein. Thus, the structure of poloxamer has been chemically modified to produce stronger gels while retaining the thermogelling properties.[69] This was achieved either by conjugating P407 with other polymers,[56,59,70–72] or by introducing inter-micelle

linkers to the poloxamer ends.[64,71,73,74] However, the change in the chemical structure would result in the formation of a novel excipient and requires additional expensive and time-consuming clinical trials to gain regulatory approval as the safety risks are unknown. Thus, it is imperative that these novel macromolecules demonstrate enhanced properties to the constituent P407 to warrant this kind of financial support. The nature and effect of these modifications is discussed in this section.

3.1. Conjugating P407 to other polymers, and polymerisation from P407 terminal groups

Covalently linking other polymers to poloxamer could lead to a thermogel with improved properties. Conjugation of P407 to chitosan, a biodegradable polysaccharide with mucoadhesive properties, has been reported by *Park et al.* [70] Chitosan-poloxamer (CP) was synthesised by carboxylation of P407 using succinic anhydride. The resulting monocarboxylated poloxamer was linked with chitosan via carbodiimide coupling, as shown in (Figure 4).[70]



Chitosan-P407 Co-polymer

Figure 4: *Synthesis of Chitosan-P407 co-polymer using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide(NHS). Image taken from information provided in* [70].

CP was thermosensitive and had improved mechanical strength when compared to P407. 16 and 20 % w/w CP solutions exhibited gelation at 25 and 15 °C, respectively.[70] The gelation temperature dropped from 21~25 °C for P407 20 % w/w to 15 °C for the 20 % w/w CP solution, which is less favourable for biomedical applications as the medicine may require refrigerated storage. From the literature, the storage modulus of a P407 20 % w/w thermogel is around 10 kPa at body temperature.[70] The CP thermogel had a storage modulus of 20 and 40 kPa for CP 16 and 20 % w/w, respectively,[70] meaning that the rheological properties were improved for the CP gels compared with P407 alone.[70] Furthermore, the storage moduli of the CP 20 % w/w thermogel was higher than the CP 16 % w/w gel which indicates the higher CP polymer concentration, the stronger the mechanical properties.[70] These outcomes displayed that the conjugation of P407 to chitosan improved the strength of the thermogels.[70] However, the

effect of the CP concentration on the gelling temperature and mechanical properties is rather limited in the study. The study also demonstrated that the gels appear to be non-cytotoxic to chondrocytes. Further evaluation using toxicological tests recognised by regulators could assist in translating this prospective excipient into clinical treatments.

Ruy et al.[59] investigated the effect of mixing catechol conjugated chitosan with thiolated P407.[59] 3,4-dihydroxyhydrocinnamic acid was conjugated to the primary amine residues in chitosan to introduce catechol pendant groups. This catechol-chitosan polymer (CHI-C) was then mixed with thiolated P407 (P-SH),[59] synthesised by coupling the P407 terminal hydroxyl groups with cystamine.[59] It was found that CHI-C alone exhibited gelation under basic conditions, forming a gel with an elastic modulus value of ∼1.0 kPa after 48 h of incubation at room temperature or 6 h at 37 °C. [59] CHI-C was blended with P-SH, to produce a fast-gelling solution. To prepare a gel of CHI-C/P-SH, each polymer was separately dissolved, then mixed for 1 h at 4 °C and 37 °C. The elastic modulus value of the formulation 0.5/12 % w/w (CHI-C/Plu-SH) thermogel was 20.8 kPa after 48 h at 37 °C and 1 Hz, which was much higher than its value for 1 % w/w CHI-C , 943.0 Pa, and for 20 % w/w P407, which was less than 10 kPa.[59,70] This difference in the elastic modulus indicates that the CHI-C/P-SH mixture has enhanced P407 gel strength. This is due to the addition of multiple catechol groups onto the chitosan backbone, which have cross-linked chitosan macromolecules in an inter- and intra- molecular fashion, as well as forming interactions between chitosan and the thiolated P407 copolymer. The catechol group loses two electrons and two protons in the basic environment, subsequently turning into quinone form, which is highly reactive with thiol and amine groups via Michael-type addition or Schiff-base formation reactions.[59] This polymer mix of CHI-C/P-SH, could be a novel tissue-adhesive thermogel with improved mechanical properties and stability.[59] CHI-C/P-SH increased the resistance of P407 gels to dissolution. Gel solutions made of 16 and 12 % w/w CHI-C/P-SH maintained 97.5 % and 75.0 % of their

weight after 25 days, respectively, following their injection into a mouse. The control (16 % w/w P407) gel fully degraded after 24 h.[59]

P407 has also been covalently bound to hyaluronic acid, an anionic nonsulfated polysaccharide belonging to the glycosaminoglycans family. Hyaluronic acid is typically made up of over 30,000 repeating polymerised units of two basic sugars units, glucuronic acid and N-acetylglucosamine [75]. First, the hyaluronic acid was conjugated with dopamine, then mixed with thiolated P407 (P407-SH) to produce a copolymer with a low degree of crosslinking (HA-P407), Figure 5. [71] Despite being chemically cross-linked, this copolymer exhibited sol-gel behaviour at the concentrations investigated, which the authors attribute to a low degree of cross-linking.

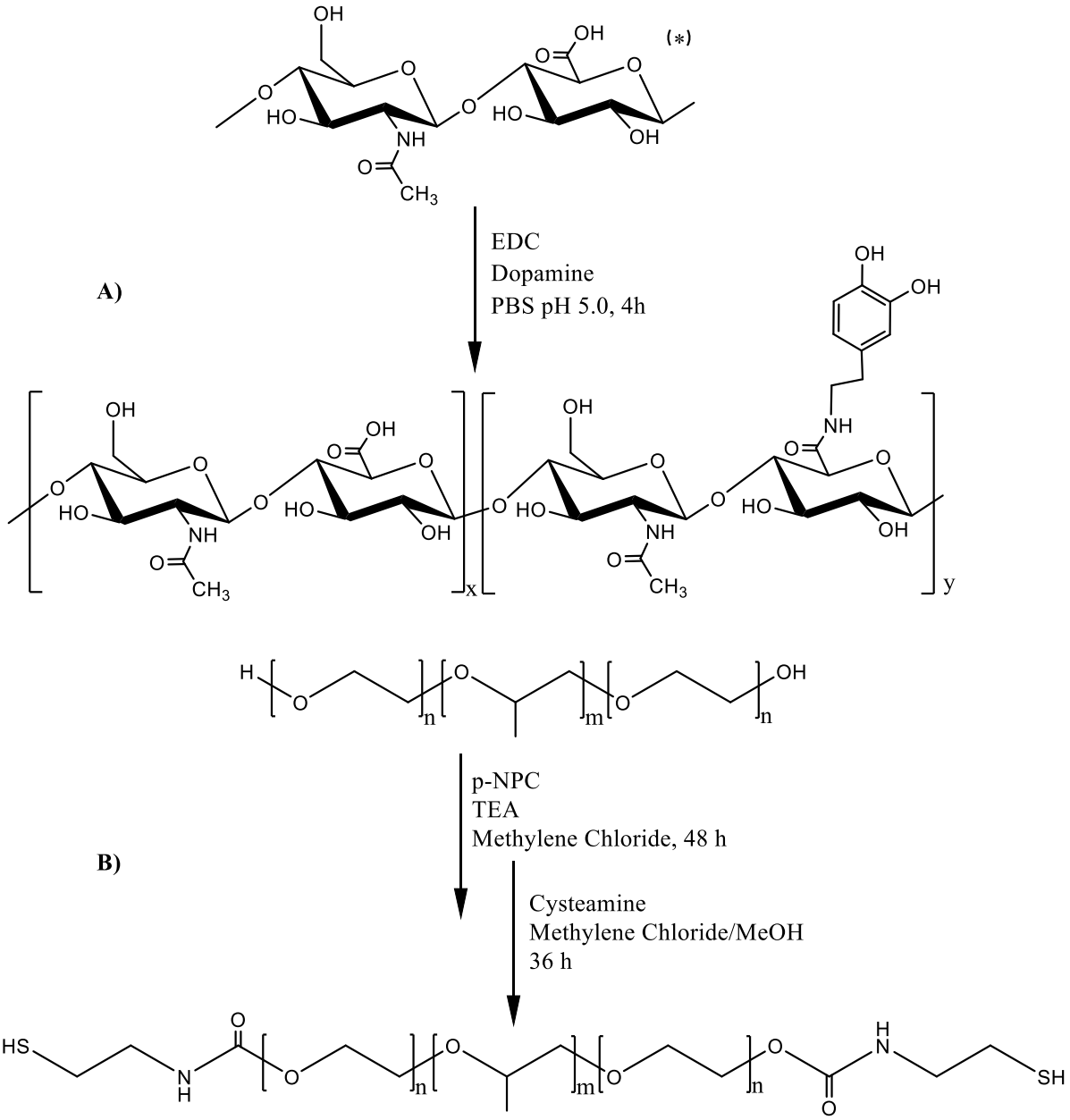


Figure 5: *Synthetic steps to a) dopamine modified hyaluronic acid (HA) using 1-ethyl-3-(3-dime- thylaminopropyl)-carbodiimide hydrochloride (EDC) in Phosphate-buffered saline (PBS) solution and b) thiolated poloxamer 407 using p-nitrophenyl chloroformate (p-NPC) and Triethylamine (TEA)* [71]

(*) Please note that the structure of HA has been simplified for clarity

HA-P407 solution exhibited significantly different sol-gel behaviour from P407. The critical gelling concentration for the HA-P407 was 7 % w/w, while for P407 was 16 % w/w. This shift

could be due to the intra-polymer network caused by the chemical cross-linking between thiol end groups in P407 micelles and the catechol groups of HA.[71] Although the authors have demonstrated that HA-P407 solution was able to form a gel at 13.8% w/w concentration while the 13.8% w/w P407 would not, a comparison with P407 at concentrations above the CGC is not made. The 13.8 % w/w HA-P407 thermogels achieve a G' of ca 10 kPa at 37 °C, comparable to a 20 %w/v P407 solution. The HA-P407 gel's displacement with applied force was measured to determine bioadhesion on rat skin. The adhesion strength of HA-P407 was 414 % more than a mixture of P407 and HA. The catechol groups have a key role in this increase, because the catechol-modified hyaluronic acid showed a 168 % increase in tissue adhesion relative to the unmodified polymers. In addition, the interaction between the polymers and mucin surface was measured by adding the polymers to mucin solution and measuring change in the elastic modulus (G`). The change in G` upon mucin addition for HA-P407 formulation was 4.4 kPa, a greater than 10-fold change in G` compared to a hyaluronic acid and poloxamer mixture, which was 0.4 Pa. The author suggests that the strong interaction between HA-P407 and mucin is the major factor in enhanced tissue-adhesion.[71]

Dou et al. have explored different approaches to modify poloxamer's structure by incorporating the polymer with polyhedral oligosilsesquioxane (POSS).[56] The P407-POSS conjugate was synthesised by a two-step process. First, P407 active ends were functionalised with an atom-transfer radical polymerisation initiator, producing Br-P407-Br, then methacrylisobutyl polyhedral oligomeric silsesquioxane (MA-POSS) was polymerised at the two ends of the Br-P407-Br to create a pentablock terpolymer (Figure 6).[56]

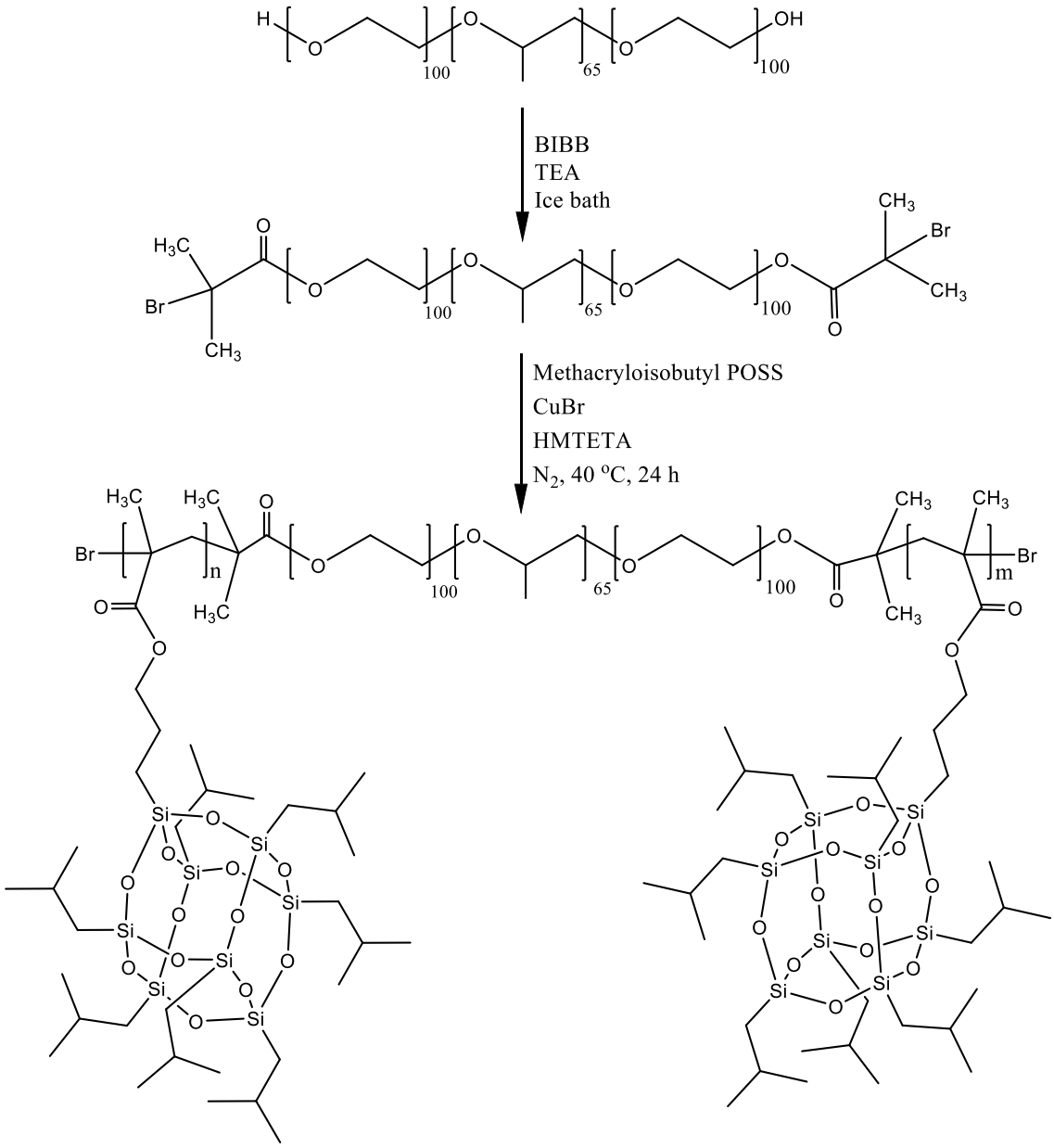


Figure 6: *Synthesis steps for P407-POSS using 2-Bromo-2-methylpropionyl bromide (BIBB), triethylamine (TEA) and 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA)* [56]

A temperature ramp from 20 to 45 °C, under constant strain of 1.25 %, and angular frequency of 10 rad·s^{-1} was performed to determine the transition temperature of a P407 and P407-POSS solution (15 % w/w each). The results showed an increase in the transition temperature from approximately 23.5 °C for the P407 solution to 33.5 °C for the P407-POSS.[56] It has been suggested by the authors that the rigid POSS block disturbs the assembly of the P407 hydrophobic segments which causes this increase in Tgel.

Cohn et al. enhanced the mechanical properties of poloxamer by the bulk polymerization of P407 with hexamethylene diisocyanate (HDI) resulting in $[P407\text{-}HDI]_p$, where p is the polymerisation degree.[72] This was achieved by mixing a 1:1 molar ratio of P407 flakes and HDI with the addition of 0.2 g of Tin(II) 2-ethylhexanoate ($SnOct_2$) as a catalyst at 80 °C for 30 min under dry nitrogen atmosphere. Also, different degrees of polymerisation were obtained by changing the HDI/P407 ratio (1.9, 3.1 and 4.1 degree of polymerisation for HDI/P407 molar ratio of 2.2, 1.1 and 1, respectively).[72] $[P407\text{-}HDI]_4$ modification dramatically increased the solution viscosity at 37 °C 15-fold for the 17 % w/w (200,000 Pa.S) solution compared with P407 at the same concentration (13,000 Pa.S). It was found that the higher the DP, the greater the mechanical properties of the gel. For example, P407, $[P407\text{-}HDI]_2$, $[P407\text{-}HDI]_3$ and $[P407\text{-}HDI]_4$ 14 % w/w solutions reached viscosities of 2, 5400, 49000 and 95000 Pa.S respectively, at 37 °C.[72]

3.2. Introducing inter-micelle linkers to the terminal groups of P407

Lee et al. synthesised a new class of injectable poloxamer thermogels with improved mechanical properties at body temperature.[64] This was achieved through enzyme-mediated crosslinking of the poloxamer micelles. P407 was conjugated with tyramine on both terminals (P407-Tyr) so that these micelles would be able to further cross-link with tyrosinase treatment (Enz-P407-Tyr) forming a highly robust gel structure (Figure 7). [64]

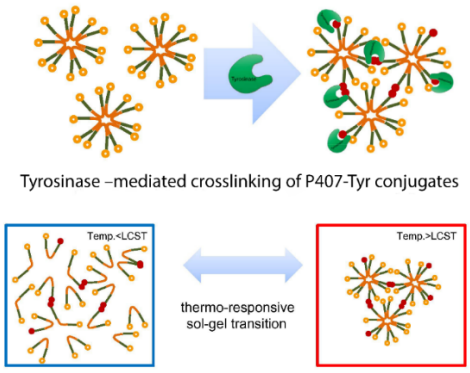


Figure 7: *Enzyme-mediated crosslinking between poloxamer micelles by conjugating the P407 with tyramine on both terminals and then crosslinking the conjugated P407 (P407-Tyr) with tyrosinase, an enzyme forming (Enz-P407-Tyr)* [64]. *Modified from Acta Biomaterialia, 7 /4, Soo Hyeon Lee,Yuhan Lee,Sang-Woo Lee,Ha-Yeun Ji,Ji-Hee Lee,Doo Sung Lee,Tae Gwan Park, Enzyme-mediated cross-linking of Pluronic copolymer micelles for injectable and in situ forming hydrogels, 1470. Copyright (2010), with permission from Elsevier.*

The authors found that tyrosinase treatment lead to dimerization of P407, and it was suggested that these dimers could introduce inter-micelle bridges between poloxamer micelles. This modification significantly changed the sol-gel transition compared with poloxamer and P407-Tyr solutions. The critical gelation concentration (CGC) for the P407 and P407-Tyr solutions were 15 and 19 % w/w, respectively, suggesting minor modification to the micelle packing behaviour. While for the Enz-P407-Tyr the CGC was only 4 % w/w,[64] which means that a minimal amount of polymer is needed to form a thermogel at body temperature.

The Enz-P407-Tyr modification made the gel's mechanical strength greater than the P407 solutions. The rheological properties of 10 % w/w Enz-P407-Tyr thermogels that have been treated with 50 or 250 U. mL^{-1} of tyrosinase were tested at 37 °C using frequency sweeps from 0.1 to 10 Hz. The results showed that when increasing the enzyme concentration from 50 to 250 U. mL^{-1}, G' significantly increased 14.5-fold at 1 Hz frequency. With either concentration of enzyme, G' was significantly greater than P407-tyr alone.[64] The Enz-P407-Tyr modification also improved the gel's resistance to dissolution. 20 % w/w solution of P407, P407-Tyr and Enz-P407-Tyr was placed in a PBS solution and the mass lost from the gels was measured. P407 and P407-Tyr solutions were completely dissolved after 3 days while 30 % of the Enz-P407-Tyr remained after 13 days.[64] Furthermore, this modification showed an improvement in the material's mucoadhesion. The tyramine groups placed on the outer-layer of poloxamer micelles were altered into catechol groups in the presence of tyrosinase. The

catechol groups exhibit high mucoadhesive properties, because of the mucin–catechol interactions. To determine the mucoadhesive properties, the interaction between the formulations and mucin (a glycosylated protein produced at the mucosal surface) was examined using rheological measurement. At 5 % w/w concentration and 37 °C, mucin and Enz-Plu-Tyr separately exhibited an elastic modulus of 0.17 and 0.36 Pa, respectively. However, after mixing these two in the sol state, below the critical gelation temperature, the elastic modulus value has significantly increased to 5.46 Pa at 37 °C. This result led the author to suggest that the Enz-Plu-Tyr thermogels could be used as a mucoadhesive biomaterial suitable for drug delivery.[64]

P407 has been modified to include acidic or basic end-groups to modify the surface charge of poloxamer micelles. Carboxylic acid functional groups may be introduced to both P407 terminals through esterification. These groups become negatively charged under physiological conditions above the pKa. A single charge on each end was obtained using succinic anhydride (SA) or a triple charge on each end using multiple reactions using p-nitrophenylchloroformate, tris(hydroxy-ethyl) aminomethane, followed by SA. Ethylene diamine (EDA) and triethylenetetramine (TETA), positively charged primary amines under physiological conditions, were also used to end cap P407, (Figure 8).[73]

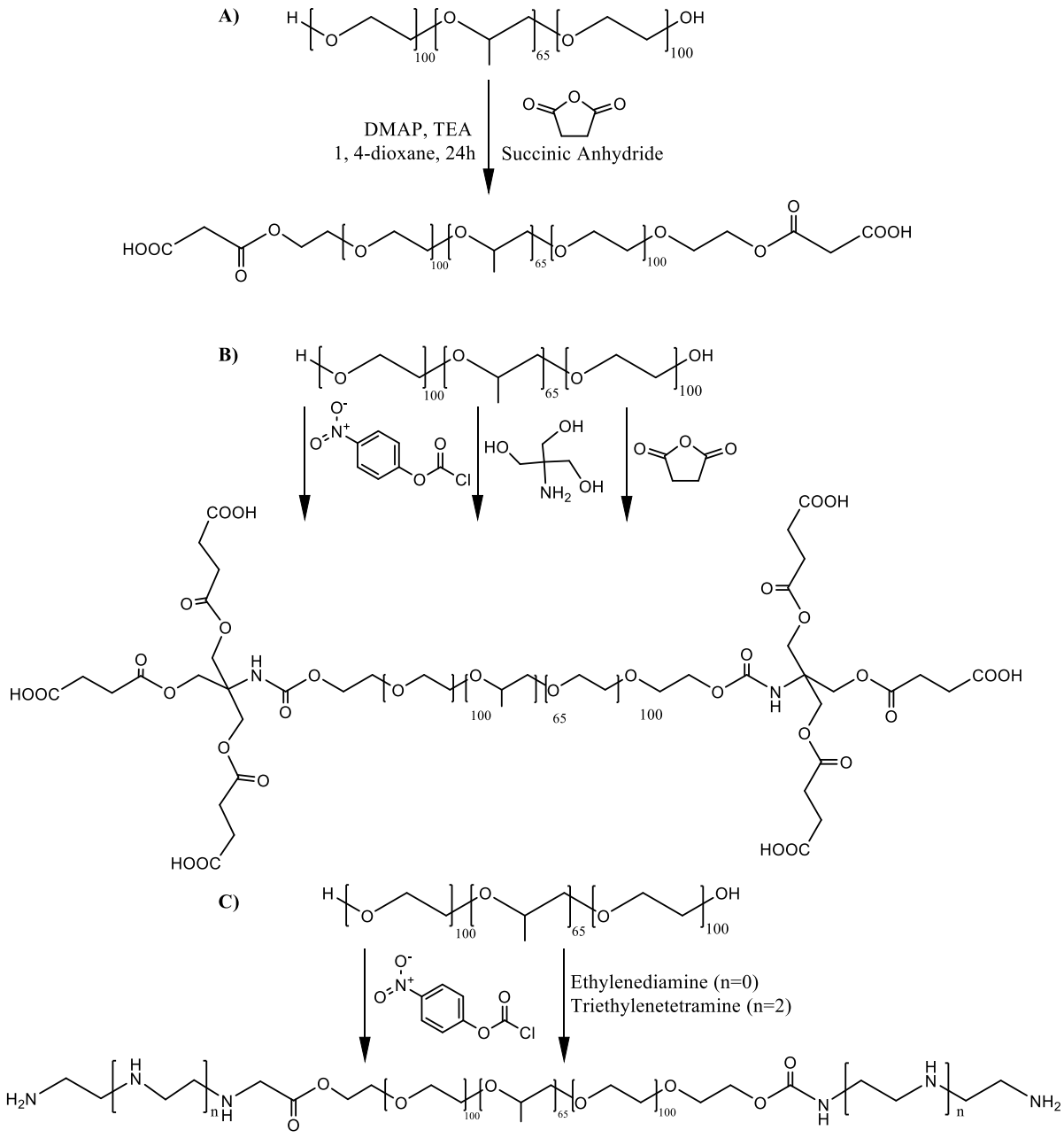


Figure 8: *Synthesis of charged P407. (A) negative charge on each end of P407 were obtained using succinic anhydride, 4-dimethylamino pyridine (DMAP) and triethylamine (TEA), (B) triple negative charge on each end using multiple reactions using p-nitrochloroformate, Tris, and succinic anhydride, Finally, (C) positive charge on each end made using Ethylene diamine (EDA) and Triethylenetetramine (TETA).* [73]

Five samples were prepared for the temperature sweep analysis at 16 % w/w total polymer content, P407, P407-SA, P407-TETA, P407-SA, P407-TETA (1:1 w/w) blend and (P407-Tris-SA)- (P407- EDA) (1:3 w/w) blend. As shown in (Figure 9), all samples showed liquid like behaviour with low G` values at temperatures lower than 20 °C, then gelation was observed by the increase in G` with temperature. On their own, at 37 °C, P407-SA and P407-TETA showed lower G` values than P407 without modification. This might be because of the repulsive inter-molecular forces, triggered by the molecule's identical charges, which make the process of micellization and packing less favourable. On the other hand, the blend of oppositely-charged polymers showed a higher G` than non-functionalised P407, which were approximately 40,000 Pa, 90,000 Pa and 170,000 Pa (900,000 and 1,700,000 dynes/cm^2) for P407, P407-SA and P407-TETA (1:1 w/w), and P407-Tris-SA and P407- EDA (1:3 w/w), respectively, at 1 Hz. This increase was attributed to the opposite charges on the polymers leading to ionic interaction between P407 micelles, which enhanced the blend's rheological properties. In addition, it was also noticed that the inter-micellar interaction increased with the increase of charges at the P407 end because the G` from the P407-Tris-SA and P407- EDA (1:3 w/w) blend was almost twice as great as the single charged P407-SA and P407-TETA (1:1 w/w) blend. [73]

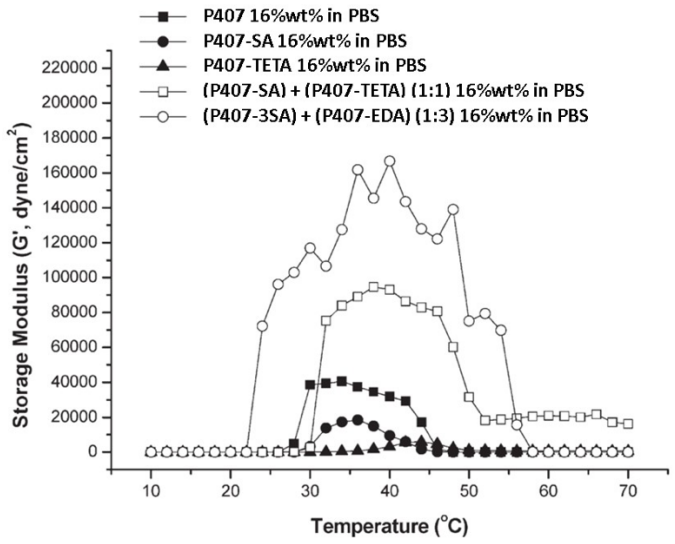


Figure 9: *Temperature ramps which illustrates that at 37 °C, P407 without modifications on its own showed higher G` values than P407-SA and P407-TETA, the blend of oppositely-charged polymers (open symbols) showed a higher G` than unfunctionalised P407 mixture.* [73] *Copyright © 2010, John Wiley and Sons*

Stereocomplexation to form crystalline domains between P407 micelles has also been explored to modify poloxamer properties.[76] *Park et al.* have investigated the effect of linking P407 copolymer end-caps at both terminals with oligo(D-lactic acid) or oligo(L-lactic acid), resulting in P407-DLA or P407-LLA, respectively. These polymers were then mixed in a 1:1 ratio, [74] which resulted in self-assembly and the formation of a "stereocomplexed thermogel" (ST).[74] P407, P407-DLA and ST showed sol-to-gel behaviour upon warming. The modified polymers, P407-DLA and ST, copolymer showed higher critical-gelation concentrations (approximately 21 and 22.5 % w/w respectively) compared to P407 alone, 18.5 % w/w, which may be due to the prevention of micelle packing caused by the presence of oligo lactide chains on the end terminals. Therefore, a higher polymer concentration would be needed for a gelation temperature comparable with P407.[74]

Chung et al. [69] have investigated the same concept of stereocomplexed hydrogels by synthesising a series of multi-block copolymers (MD_n or ML_n) formed from P407 linked by D-lactide or L-lactide oligomers with different spacer lengths, and a diisocyanate compound as crosslinker (Figure 10).[69] The two optical isomers (MD_n and ML_n) were blended in a 1:1 ratio to form a stereocomplexed thermogel. The enantiomeric poly(L-lactic acid) and poly(D-lactic acid) formed a robust stereocomplexed crystalline structure.[69]

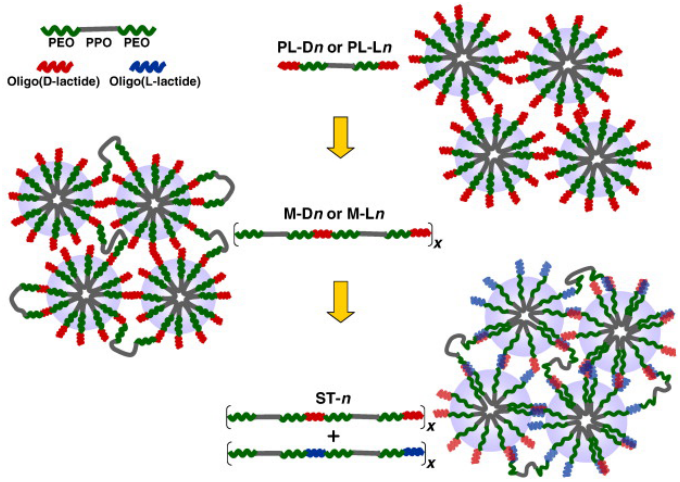


Figure 10: *Molecular structure of P407 - MDn and MLn and the formulation of MST. Reprinted from Journal of Controlled Release, 127 /1, Hyun Jung Chung,Yuhan Lee,Tae Gwan Park, Thermo-sensitive and biodegradable hydrogels based on stereocomplexed Pluronic multi-block copolymers for controlled protein delivery, 23., Copyright (2007), with permission from Elsevier.* [69]

Mixing MD_n and ML_n gave physically crosslinked P407 hydrogels with significantly lower critical gelation concentrations and temperatures, compared to the uncomplexed multi-block or P407 solutions. These significant changes were dependant on the chain length of oligo(lactic acid). For oligomers with a degree of polymerisation of 6, the stereocomplexed hydrogels showed higher critical gelation temperatures and concentrations compared with P407. This might be due to oligo(lactic acid) chains length not having sufficient length for stereocomplexation and possibly disturbing the formation of an ordered micelle packing structure. This could also result in a higher polymer concentration required for gelation. In contrast, when n = 12 and 18, stereocomplexed hydrogels had significantly lower critical gelation temperatures and concentrations compared to the P407. The gelation temperature ranges were also broader than the corresponding multi-block hydrogels. Thus, it can be concluded that gelation of the mixed hydrogels occurred at lower temperatures with a longer oligo(lactic acid) spacer as well as at much lower polymer concentrations. Specifically,

mixing resulted in gel formation at 12.5 % w/w for DP=12 and 8.75 % w/w for DP=18, comparing to ~16 % w/w for P407 alone. [69]

The multi-block ML, stereocomplexed ML/MD blend, and P407 gel strength were tested by running oscillation frequency ramps on a rheometer. All three formulations were prepared at 17 % w/w concentration at 37 °C and the storage modulus (G') and loss modulus (G") values were measured. The MD/ML gel blend exhibited significantly higher elasticity (G'=26,100 at 10 Hz), 8.8-fold more than P407 (G'=2,980 at 10 Hz). Also, ML formulation exhibited 5.5-fold higher G' values compared to P407. Thus, stereocomplexed MST structures have greatly enhanced the mechanical strength of the P407 formulation because of the increase of the crosslinking density.[69]

3.3. Summarising the effect of covalent modification on the thermogelling behaviour of P407

In summary, the covalent modification of poloxamer has shown to be an effective method of improving the gel properties significantly, as well as significant reduction in the polymer concentration required to form a gel. The results are summarised in Table 2, but please note that simplifications must be made and the original papers should be referred to for a full understanding. It can be seen that the majority of studies achieve gels with G’ greater than the 10 kPa typical for a 20 % P407 solution. In particular, modifying the end groups of P407 to promote adhesive interaction between micelles appears to produce enhanced gel strength. There is a need for the studies to elaborate on mechanisms for these improvements, supported by analytical evidence, so that future research has the strongest possible foundations to design advanced materials. Furthermore, these modifications would produce materials with unknown safety profiles, and the reported studies do not cover the battery of toxicological tests which would be required by regulators. We suggest that future research aligns with these requirements to produce materials which are more easily translated into the clinic.

Table 2: *a summary of the effect of P407 chemical modification on thermogelling behaviour. N/A used where value was not reported in the article (at the reported concentration)*

Chemical modification						
Conjugating P407 to other polymers						
Modification	Concentration	Tgel / °C		Gel Strength		References
		P407	Modified	P408	Modified	
Chitosan-P407	20 % w/w	21-25	15	10 kPa[a]	40 kPa[a]	Park et al. [70]
Catechol conjugated chitosan with thiolated P407 (Plu-SH)	CHI-C/Plu-SH 0.5/12 % w/w	N/A	28.6	N/A	21 kPa[a]	Ruy et al.[59]
hyaluronic acid with thiolated Plu-SH	HA/Plu-SH 5/13.8 % w/w	N/A	19	N/A	Ca 10 kPa[a]	Lee et al. [71]
Polyhedral oligosilsesquioxane-modified P407	15% w/w	23.5	33.5	0.1 kPa[a]	0.1 kPa[a]	Dou et al. [56]
P407 end group modification with hexamethylene diisocyanate [P407-HDI]p	17% w/w	Ca. 25	Ca 22	13 kPa.s[b]	200 kPa.s[b]	Cohn et al. [72]
P407 with acidic/basic end groups	16 % w/w	27	22	20 kPa[a]	170 kPa[a]	Lee et al. [73]
Enzyme-mediated crosslinking of tyramine-capped P407	10 % w/w	Below CGC	Ca. 22	N/A	Ca 2 kPa[a]	Lee et al. [64]
Modification of P407 end groups with crystallisable lactide oligomers	17% w/w	N/A	N/A	3 kPa[a]	26 kPa[a]	Chung et al. [69]

a: storage modulus (G`)
b: viscosity
note: the different values of G` and Tgel for the P407 at the same concentration is because of the parameters which the gel been tested (stress, frequency and temperature rate)

4. Poloxamer 407 modification by the use of polymer additives

The addition of hydrophilic polymers to aqueous solutions of poloxamer 407 has been reported to enhance the thermogel's properties. These additives include crosslinked polyacrylic acid, polyvinyl alcohol and polysaccharides. Nanoparticles have also been investigated. The advantage of this mixing method is that the chemical structure of P407 is unchanged (assuming no covalent interaction between polymers), and thus carries a lower risk of causing adverse effects *in vivo* than covalent modification. Pharmaceutical excipients known to be well-tolerated in humans are the preferred choice for addition to P407 solutions, ensuring a biocompatible dosage form. In this section we discuss the effect of polymer addition on P407's properties, including Tgel, rheology, and mucoadhesion. This section is separated by the additives used.

4.1. The addition of different poloxamer types:

Mixing other grades of poloxamers (PEO-PPO-PEO) with P407 results in changes in the transition temperature. *Kim et al.* have studied mixtures of poloxamer 407 and 188 by fixing the concentration of P407 at 12, 14, 16 and 20 % w/w, and changing the concentration of P188 from 10 to 30 % w/w for each concentration.[25] The gelation temperature decreased when increasing the concentration of both P407 and P188.[25] For example, solutions prepared using P407/P188; 12/10, 12/20 and 12/30 % w/w showed a Tgel of 55, 38 and 23 °C, respectively, while solutions with P407/P188; 20/10, 20/14 and 20/20 had Tgel at 27.5, 24 and 15 °C.[25] The authors did not provide an explanation for this phenomenon, but it is possible that increasing overall solute concentration could lead to a reduction in Tgel. It is known that P407 and P188 do not form mixed micelles, so this is not a possible explanation for the difference in Tgel.[77] In another study, *Xuan et al.* showed that adding increasing amounts of P188 to 15 % w/w P407 solutions, increased the viscosity of the mixture at 25 °C.[33] The study results

showed that when 15 % w/w of P188 was added to 15 % w/w P407 the solution viscosity at 25 °C was 151-152 mPa.s and when the P188 concentration increased to 17, 18 and 20 % w/w the solution viscosity increased to 178-179, 200-202 and 232-235 mPa.s, respectively.[33] In another study, P188 10 % w/v was blended with 10 % w/v P407 and the effect of this addition was compared with 20 % w/v P407.[75] Tgel for the blend was 33 °C, 6 °C above the Tgel for P407 solution. Also the G` for the blend was lower than the P407 solution (6,410 and 10,600 Pa respectively).[75] This may be partly due to the decrease in P407 concentration in the blend which in turn which caused the increase of Tgel and decrease of G`. Al Khateb and coworkers investigated the *ex vivo* retention of P407 20 % w/v, P188 20 % w/v and a 10:10 % w/v mixture of the two. It was found that the 20% w/v P407 solution had the greatest retention on *ex vivo* bovine cornea. It was also found that the *in vivo* retention of fluorescein in P407 20% w/v solution on rabbit eye was greater than fluorescein in 20% w/v P188. Only the P407 preparations formed gels at the temperatures tested.[78]

4.2. The addition of Carbopol® (C934P and C971P) to Poloxamer 407:

Carbopol® is a crosslinked polyacrylic acid polymer which comes in different grades depending on the crosslinking density and the polymerisation solvent used. Carbopol 934P (C934P) and 971P (C971P) are two types of Carbopol® commonly added to P407. They have the same crosslinking density, but with benzene or ethyl acetate used as polymerisation solvent, respectively.[79] They have been extensively studied as an additive to poloxamer formulations due to their mucoadhesive properties which would be advantageous for mucosal drug delivery, but they have also been reported to modify other properties of the gel.[7,54] *Jones et al.*[60] have investigated the addition of C934P to P407 solutions, and found that the Tgel increased from 31 to 35 °C when 0.1 % w/w C934P was added to a 15 % w/w P407 solution. However, Tgel then started to decrease to 32.5 ± 0.5 °C and 31.9 ± 0.8 °C with increasing C934P concentration from 0.15 % w/w to 0.25 % w/w, respectively. The addition of 0.1 % w/w C934P

to poloxamer 407 20 % w/w solution reduced Tgel from 25.4 ± 0.1 °C to 23.4 ± 0.4 °C which continued to fall with C934P concentration to 21.8 ± 0.4 °C at 0.25 % w/w C934P.[60] It was also found that the incorporation of C934P increased the mucoadhesion of the poloxamer solution. Solutions containing 15 % w/w P407 did not exhibit measurable mucoadhesion properties until 0.2 and 0.25 % w/w of C934P was added. 20 % w/w P407 solutions exhibited a measurable force of mucoadhesion, and adding C934P increased mucoadhesion until 0.2 and 0.25 % w/w, which had the same mucoadhesion force of 0.39 N.[60] *De Souza Ferreira et al.* have investigated the addition of Carbopol 971P (C971P) to P407 solutions.[54] For solutions containing 15 % w/w P407, the addition of 0.1 % w/w C971P increased Tgel from 31.5 °C to 39.7 °C, which then increased to 41.1 °C at 0.15% w/w C971P. After this point, the gelling temperatures dropped with an increase of C971P concentration to reach 28.3 °C at 0.5 % w/w of C971P.[54] Similarly, when using 20 % w/w P407 solutions, the highest Tgel was reached when adding 0.25 % w/w C971P which increased the gelling temperature from 25.4 °C to 28.8 °C, while the addition of 0.5 % w/w dropped the Tgel to 24.1 °C.[54]. The author's noticed that low Carbopol® content was enough to form an interaction with P407, these were believed to be hydrogen bonds between the hydroxyl groups of P407 and carboxyl groups in acrylic acid. However, when the concentration of Carbopol® increased to 0.50 % w/w the Tgel dropped for both P407 solutions. The authors suggest this may be due to competition between Carbopol® chains to interact with P407, and the interaction between Carbopol® polymers and themselves.[54]

Majithiya et al. examined the addition of C934P to an 18 % P407 solution and studied the formulation's suitability for nasal drug delivery.[26] They found that the transition temperature of 18 % w/v P407 solutions decreased with an increasing concentration of C934P, with 0, 0.1, 0.2, 0.3 and 0.5 % w/v C934P giving a Tgel of 28.2, 27.3, 25.8, 24.8 and 23.0 °C, respectively.[26] This is in contrast to Jones *et al.* who found that 0.1 % w/w addition of C971P

to 15 % w/w P407 solution increased Tgel. Jones *et al.* did, however, see a similar trend when adding Carbopol to 20% P407 solutions. This indicates that at lower P407 concentrations, a small concentration of carbopol (0.1 % w/w) increases the gelling temperature, however, at higher P407 concentrations this effect is not seen. *Majithiya et al.* also found that the mucoadhesive strength of 18 % w/v poloxamer 407 formulations increased with C934P concentration. However, The change wasn't significant at 0.1 % w/v C934P comparing to P407 alone, but when raising the C934P concentration to 0.2, 0.3 and 0.5 % w/w the mucoadhesion was significantly increased with the increase of C934P.[26]

El-Kamel et al. studied the addition of 15 % poloxamer P188 or 1 % methylcellose (MC) to a 20 % poloxamer P407 and 0.5 % C934P solution (the authors did not specify % w/w or % w/v). Both additives increased Tgel from 23 to 30 °C.[53] In addition, the solution containing 15 % P188 along with C934P 0.5 % and 20 % P407 showed the highest gel strength compared with the other two solutions. However, although the authors achieve their target Tgel, the study is not sufficiently systematic to understand the effect of polymer concentration on Tgel.

4.3. The addition of polycarbophil:

Polycarbophil (also named Noveon®), is high molecular mass poly (acrylic acid) cross-linked with divinyl glycol, and has also been investigated as an additive to P407.[80] Polycarbophil has been used as an inactive ingredient in medicines approved by the FDA, and shows no irritation to the skin or mucus surfaces, as well as having strong mucoadhesive properties which makes it a good candidate as an additive to poloxamer formulations.[80] *De Souza Ferreira et al.* have investigated various formulations using polycarbophil (0.10, 0.15, 0.20, 0.25 or 0.50 % w/w) with P407 (15 or 20 % w/w). The addition of polycarbophil lead to an increase in the transition temperature, from 31 °C to 39 °C for 15 % w/w P407 solution alone or with 0.1 % w/w polycarbophil, respectively, and from 25 °C to 28 °C for 20 % w/w P407 solution alone or with 0.1 % w/w polycarbophil, respectively. The 20 % w/w P407 solution Tgel appeared to

plateau as polycarbophil concentration was increased from 0.1 to 0.5 % w/w. However, the Tgel of 15 % w/w P407 decreased from 36.4 ± 0.05 to 26.9 ± 0.2 °C as the concentration of polycarbophil was increased from 0.25 to 0.5 % w/w. The increases in Tgel were attributed to the hydrogen bonding between the polycarbophil and P407 hydrophilic segments (PEO), interrupting the self-assembly of micelles, and therefore gel formation.[80] There was no linear correlation between the transition temperature and polycarbophil concentration.[80] Polycarbophil is also a useful additive to the P407 solution to improve mucoadhesion. In theory, increasing the concentration of the mucoadhesive polymer (polycarbophil) should increase the force of mucoadhesion. However, solutions containing 0.15 and 0.20 % w/w of polycarbophil exhibited significantly higher mucoadhesion force than 0.25 and 0.50 % w/w solutions. The authors suggest that this might be due to the greater amount of polycarbophil leading to increased shielding of free hydroxyl groups in P407 to interact with the mucosal surface.[80] As in the case of Carbopol®, increasing P407 concentration from 15 to 20% w/w significantly increased the force of mucoadhesion.[80] In addition, polycarbophil and P188 have been studied in combination as additives to increase the mucoadhesion of poloxamer. 15-20 % w/w of P407 and 15-20 % w/w P188 were used along with 0.2, 0.4 and 1.0 % w/w of polycarbophil. It was found that increasing the percentage of P188 or polycarbophil significantly increased the mucoadhesive strength.[28]

4.4. The addition of Poly(vinyl alcohol) to poloxamer 407 solutions

Poly(vinyl alcohol) (PVA) has also been investigated as a potential additive to P407 solutions. PVA repeat units contain hydroxyl groups which could lead to cohesive hydrogen bonding. Five formulations of P407 and PVA have been studied and prepared by *Bercea et al.* The formulations were prepared at a constant polymer content of 20 % w/w with different PVA/P407 ratios (20:0, 15:5, 10:10, 5:15 and 0:20).[57] This would eliminate the effect of

increasing the overall polymer content in the formulation, however the results are confounded by the effect of P407 concentration on the rheological parameters measured.

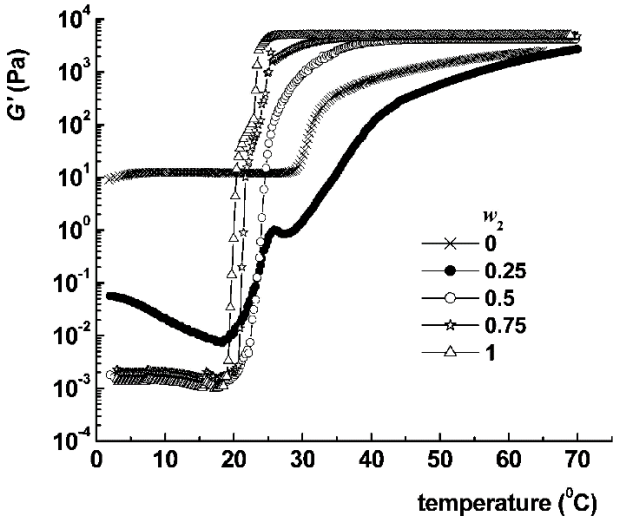


Figure 11: *Temperature ramp for the five formulations showing the storage modulus G` with the increase of the temperature. 0, 0.25, 0.5, 0.75 and 1 refer to 20:0, 15:5, 10:10, 5:15, and 0:20 % w/w ratios of PVA:P407. Reprinted (adapted) with permission from* [57]. *Copyright(2011) American Chemical Society.*

It can be concluded from temperature ramps (Figure 11) that all the samples exhibited low resistance to flow at low temperatures, except for the PVA/P407 (20:0), which is a physically entangled gel of PVA. When increasing the temperature above 19 °C, all formulations containing 10 % w/w P407 or more increase in viscosity in a narrow temperature range which indicates the beginning of the transition from sol to gel.[57] The results illustrate that the increase of PVA concentration in the formulation increased the gelation temperature which has reached 36.7 °C for PVA/P407 (15:5) solution.[57] However, it could be due to the decrease of the poloxamer content in the formulation. The mixture composed of PVA/P407 15:5 showed the highest G` value (5.9 kPa compared with 4.9 kPa for the P407 solution) which indicates that PVA improved the P407 gel strength.[57] However, with higher PVA concentrations, the G` decreased, which is likely to be due to the decrease in the P407 concentration relative to the

other formulations and it would be valuable to investigate the effect of PVA content at a fixed P407 concentration.

4.5. The effect of polysaccharides on P407 gels

Polysaccharides are sustainable, biocompatible and biodegradable naturally occurring polymers. In addition, they have diverse functional groups which made them suitable for chemical modification. [81]

A. Hydroxypropyl methylcellulose and methylcellulose as an additive to modify the properties of poloxamer solutions:

Hydroxypropyl methylcellulose (HPMC), a semi-synthetic polymer derived from cellulose, used in the food industry as thickener, emulsifier or stabilizer,[82] has been investigated as an additive to P407 solutions. Methylcellulose (MC), another cellulose derivative, has also been explored.[55]

A study by *Salem et al* examined the addition of 1 % w/v HPMC or 1 % w/v MC to 17, 25 and 30 % w/v P407 solutions. It was seen that both MC and HPMC caused Tgel to decrease, except when adding HPMC to 17 % w/v P407, which increased the temperature significantly from 29.8 °C to 48.8 °C, however, when adding MC to the 17 % w/v P407 solution, the Tgel dropped to 25.5 °C. 25 and 30 % w/v P407 had Tgel values of 23.25 and 15 °C, which dropped to 21 and 13 °C, respectively, after the addition of 1 % w/v HPMC. 1 % w/v MC addition decreased the Tgel further, to 19°C and 10.5 °C for P407 25 and 30 % w/v solutions, respectively.[55] However, the authors have not investigated the effect of HPMC and MC concentration on Tgel. From this study it was also clear that increasing the P407 concentration improves the mixture's resistance to dissolution. Solution containing 17 % w/w P407 dissolved completely after 4 hours, faster than 25 and 30 % w/w P407 gels which dissolved after 8 hours. Moreover, the addition of HPMC and MC improved the solution's resistance to dissolution, with MC addition

giving more resistance than HPMC. For example, P407 at 30 % w/w completely dissolves within 8 h, whilst 50 % and 10 % of the HPMC and MC-containing samples, respectively, dissolved during this time. [55] The authors attribute the MC samples' resistance to dissolution to the greater hydrophobicity of the additive. [55]

B. The effect of carrageenan on poloxamer gels

Liu et al found that adding 0.2 % w/w of carrageenan (a sulfated polysaccharide) to poloxamer P407 20 % w/w solution had no effect on the Tgel which was 21.6 °C and 21.7 °C for 20 % w/w P407 and P407+ carrageenan solutions, respectively. However, carrageenan addition decreased the gel's G' at body temperature which indicate that carrageenan has reduced the gel's elastic properties.[49] The authors suggest this may be due to a disruption of poloxamer micelles by carrageenan chains which would lead to interference of the gelling mechanism.[49] However, it was also found that carrageenan-containing P407 formulations had significantly reduced dissolution in comparison with the control P407 which at 20 % w/w solution dissolved completely within 6 h, whilst adding 0.3 % w/w carrageenan lead to the gel remaining unchanged over 10 h exposure to dissolution fluids.[49]

C. The effect of mixing hyaluronic acid on P407 formulation:

Formulations containing poloxamer 407 and HA have been studied to improve the rheological and mucoadhesive properties of the poloxamer thermogels.[75] A 20 % w/v solution of P407 was studied with the addition of HA at 0, 0.025 and 0.05 % w/v, which decreased the G` of the solution from 10,600 Pa for P407 alone to 6,740 Pa and 6,890 Pa after the addition of HA 0.025 and 0.05 % w/v, respectively. It also increased the Tgel from 27 °C for P407 to 29 °C for both HA concentrations.[75]

Various concentrations of HA have been investigated as additives to formulations containing both P407 15% w/w and P188 10 % w/w.[83] HA was found to increase G` with the increase of HA concentration. At 43 °C and 0.5 Hz G` for the solutions prepared were 137, 174, 990,

4300 and 7300 Pa for solutions containing 0, 0.5, 0.8, 1.0 and 2.0 % w/w of HA, respectively.[83] The effect of HA on the poloxamer formulations was further studied using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) which showed a decrease in the number of water molecules interacting with the poloxamer and a decrease in the water evaporation rate with the increase of HA, respectively.[84] This suggests that HA addition interferes with the water/poloxamer interaction which makes interactions between poloxamer molecules more favourable.[84] Also, photon correlation spectroscopy analysis showed formulations with HA present have aggregates higher in dimensions than the poloxamer micelles, which indicates a secondary bond interaction between HA and poloxamer (Figure 12) [84] which might explain the increase in the G` values with the increase of HA concentration.[84]

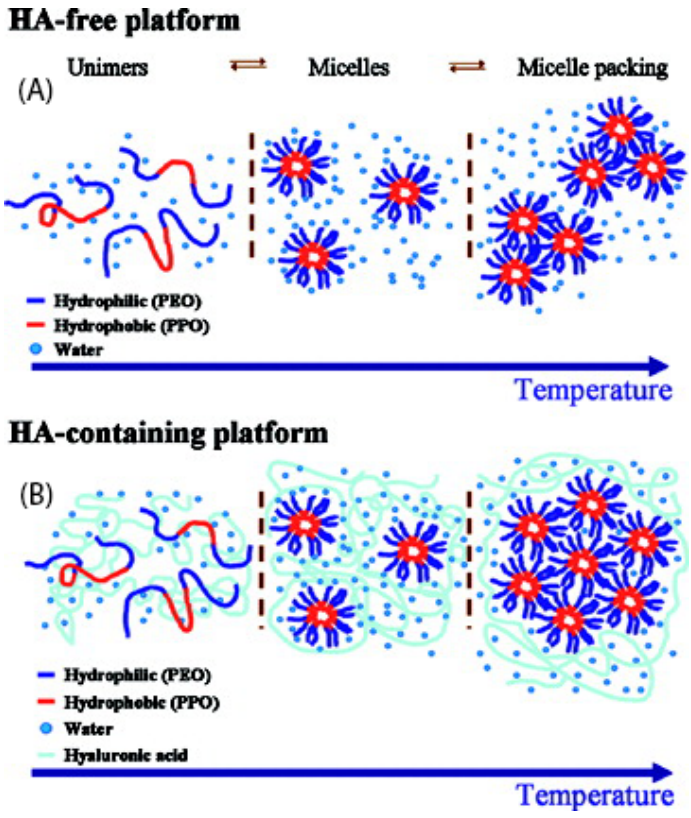


***Figure 12**: micellation and gel formation of P407 solutions with (A) and without HA (B) with the increase of temperature. Reprinted (adapted) with permission from* [81]. *Copyright (2011) American Chemical Society*

D. The effect of chitosan on P407 gels

Chitosan is a biodegradable polysaccharide with mucoadhesive properties.[85] This is due to its chemical structure that contains positively charged amine groups that interact with the negatively charged mucous surface by electrostatics, which are supported by hydrophobic interactions.[86,87] Thus, the addition of chitosan to poloxamer solutions is postulated to improve mucoadhesion.[88]

The addition of chitosan to 16 % w/w P407 solutions did not significantly affect the Tgel. At lower concentration, chitosan increased the Tgel from 32 °C for P407 alone to 33 °C after the addition of 0.5 % w/w chitosan. However, with further increase of chitosan concentration the Tgel reduced to 32 °C and 31 °C when adding 1 and 1.5 % w/w chitosan, respectively.[88] Increasing the amount of chitosan in the polymer solution significantly increased the mechanical properties of the gel formed and its mucoadhesion property.[88] At 35 °C, 16 % w/w P407 and at low chitosan (0.5 % w/w) concentration there was no significant change. However, when increasing the concentration to 1 and 1.5 % w/w the mechanical properties of the solution, and mucoadhesion, increased significantly (Figure 13). [88]

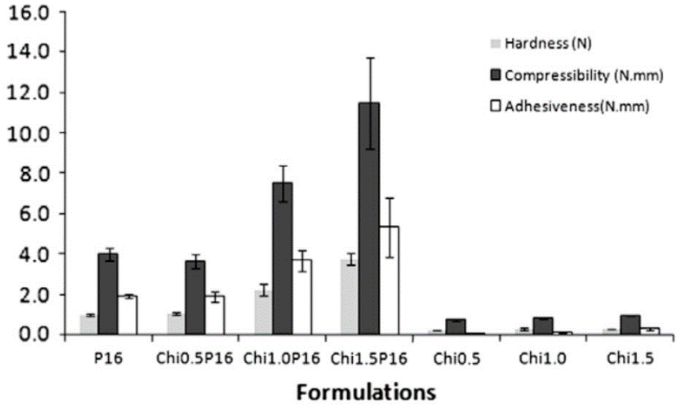


Figure 13: *physical properties of P407 formulations with and without chitosan as well as chitosan alone formulations. P16 indicates a concentration of 16 % w/w P407, whilst the numerical value following "chi" indicates the % w/w concentration of chitosan. Reprinted*

from European Journal of Pharmaceutics and Biopharmaceutics, 75 /2, Taís Gratieri,Guilherme Martins Gelfuso,Eduardo Melani Rocha,Victor Hugo Sarmento,Osvaldo de Freitas,Renata Fonseca Vianna Lopez, A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery, 190., Copyright (2010), with permission from Elsevier.

E. Alginate as additive to P407 solution:

Alginate is a polysaccharide extracted from brown algae or biosynthesised using bacteria. Alginate gel's biocompatibility and ionotropic gelation make it favourable for numerous biomedical applications including wound healing, drug delivery, as well as tissue engineering.[89] Alginate structure contains consecutive or alternating blocks of (1,4)-linked β- D-mannuronate (M) and α-L-guluronate (G) residues. The G blocks may be cross-linked by divalent metal ions, usually Ca^{2+} [89]

To investigate the effect of alginate on P407 solutions, various concentrations of alginate were added to 14 % w/w P407. It was found that a concentration of alginate lower than 0.7 % w/w was required for gels to form above room temperature.[90] It was found that the addition of 0.1 % w/w alginate to P407 led to a gel with increased viscosity, approximately double that of P407 alone at 200 s^{-1}. This was attributed to cross-linking of micelles by noncovalent interactions with alginate chains. Pilocarpine was incorporated into the formulations. It was found that P407 with 0.1 % w/w alginate administered ocularly to rabbits gave an extended pharmacological response relative to P407. [90]

4.6. The effect of nanoparticle addition to the properties of poloxamer 407 solutions

Nanoparticle medicines are extensively reported in the academic literature to achieve targeted and controlled delivery of therapeutic molecules. There are several studies investigating the incorporation of nanoparticles into P407 solutions, to combine the nanoparticles' properties with thermoresponsive gelation.

Thermogelling solutions containing tenofovir (TFV), an antiretroviral agent for the prevention of HIV acquisition, loaded into chitosan nanoparticles was prepared by Timur *et al.*[91] The P407 formulation containing chitosan nanoparticles had a Tgel of 26.6 °C which is slightly lower than the P407 20% w/v formulation with TFV only which was 28.2 °C. [91] At 35 °C, formulation containing nanoparticles had higher viscosity than the gel with the TFV only (100.24 Pa.s and 87.8 Pa.s, respectively).[91]

Poly(isobutylcyanoacrylate) (PIBCA) nanoparticles coated with a mixture of chitosan and thiolated chitosan have been known for their enhanced mucoadhesion properties due to the interaction between the thiolated chitosan and the thiol groups of cysteine residues in the mucin glycoprotein coating mucosae. [92,93] In addition, these nanoparticles present anti-Trichomonas vaginalis activity without the addition of drug.[94] The effects of PIBCA nanoparticle on P407 solution were studied by adding the nanoparticles at two concentrations, 20 mg/mL and 10 mg/mL to 20 % w/w P407 solution. The addition of the PIBCA nanoparticles showed no large change of the Tgel, which was 20.8 °C for P407 without the addition and 20.6 °C and 20.2 °C for the 10 mg/mL and 20 mg/mL concentrations of nanoparticles. The addition had a negative effect on the G' values of the formulation which dropped from 20,800 Pa for P407 to 18,400 Pa and 17,100 Pa for the 10 mg/mL and 20 mg/mL nanoparticles addition, respectively.[94] The authors suggest that this is because some of the P407 chains were unable to form micelles due to interaction with the nanoparticles.[94]

Wu *et al.*[58] studied the effect of adding Laponite Silicate nanoparticles to P407 on the gel's dissolution rate. A 20 % w/w P407 solution mixed with 3 % w/w Laponite (P20L3) was compared with a 20 % P407 solution as reference. To measure the dissolution and to make it possible to differentiate between dissolved and un-dissolved gel both formulations were coloured with methylene blue. The dissolution experiments were performed at 37 °C in buffer solution. In this study, the reference sample P20 dissolved over 40 h [58] (which is long time

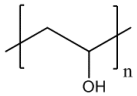
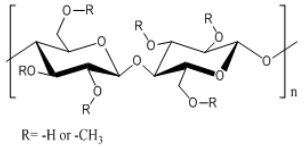
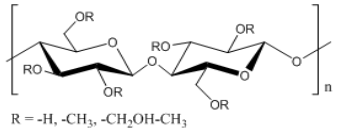
comparing with other studies [95] due to the nature of the experiment which makes the buffer contacts only the surface of the gel). On the other hand, P20L3, the sample with the nanoparticles, was stable throughout the test time of 72 h. The authors suggest that this stability was achieved because of the polymer interaction with the charged Laponite nanoparticles, but this was not confirmed experimentally.[58] Although the addition of Laponite to P407 has increased the gel stability, the 40 h dissolution time of P407 alone is unexpectedly long given its reported rapid dissolution rates. However, dissolution was measured in a vial, and only a small surface area of the gel is in contact with the buffer solution and kept steady during this period which is not the case in physiological environments. The addition of Laponite to P407 also affected the Tgel, increasing the gelling temperature from 22 °C for P20 to 26 °C for P20L3. Furthermore, the study showed that a mixture of only 4% w/w P407 and 5 or 6 % w/w Laponite exhibit a thermogelling behaviour at 51 °C and 44 °C, respectively.[58]

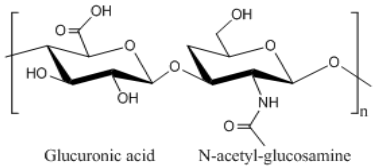
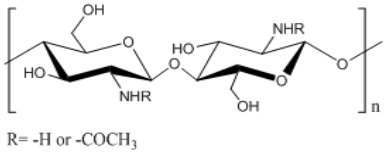
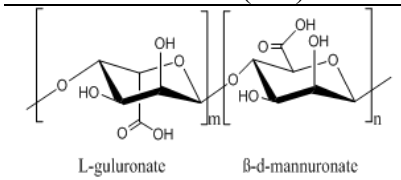
4.7. Summarising the effect of polymer additives on thermogelling poloxamer 407 solutions

The effects of polymer additives on Tgel and gel strength are summarised in table 3. Out of necessity, the findings have been simplified and the original articles should be consulted to fully understand the findings. Most studies report a decrease in Tgel when polymer or nanoparticle additives are incorporated into P407 solutions [25, 26, 53 - 55, 60, 88, 91], which may be a result of the increased overall solute content. Whilst some studies report an increase in Tgel [54, 60, 75, 80], this occurs only at lower (ca 15 % w/v) P407 concentration and low (ca < 0.1 % w/v) additive concentration. Improvements to P407 gel strength using additives are often small compared to covalent modification, and in many instances gel strength is compromised or unchanged. It is possible that the liquid crystalline nature of P407 means that the incorporation of additives results in a disruption to this structure, rather than a desirable

improvement in cohesion via intramolecular bonding. This is a hypothetical consideration, as there is a lack of mechanistic study reported in the literature, which could be supported with spectroscopic (e.g. Raman, FTIR) and scattering (e.g. small-angle neutron/x-ray scattering) techniques. There have been studies demonstrating improvements in other parameters, however, with mucoadhesive additives tending to achieve the desired effects and cellulose derivatives improving resistance to dilution. The reported studies tend to focus on binary mixtures, and the exploration of complex systems more representative of pharmaceutical formulations could be supported by recent advances in computational methods. These computational methods could also assist in optimising formulations where complex relationships occur between rheological properties and composition, for example the non-linear effects seen between additive concentration and Tgel.

Table 3: The effect of polymer additives on Tgel and gel strength

	Modification	Concentration	Tgel / °C		Gel Strength		References
			P407	Modified	P407	Modified	
Crosslinked Polyacrylic acid	Carbopol 934P	P407 20 % w/w C934P 0.15 % w/w	25	23	13 kPa[a]	16 kPa[a]	Jones et al.[60]
		P407 20% P188 15% C934P 0.5%	N/A	30	N/A	650 g[b]	El-Kamel et al. [53]
	Carbopol 971P	P407 20% w/w C971P 0.2% w/w	25	28	13 kPa[a]	17 kPa[a]	De Souza Ferreira et al. [54]
		P407 18% w/v C971P 0.2% w/v	29	26	N/A	N/A	Majithiya et al. [26]
	Polycarbophil	P407 20 % w/w PCB 0.5 % w/w	25	29	0.23 N[c]	0.42 N[c]	De Souza Ferreira et al. [81]
Polyvinyl Alcohol	 PVA	P407 15 % w/w PVA 5 % w/w	N/A	25	N/A	5.9 kPa[a]	Bercea et al [57]
Polysaccharides	 Methylcellulose (MC)	P407 25 % w/v MC 1 % w/v	23	19	630 cP[d]	794 cP[d]	Salem et al. [55]
	 Hydroxypropyl methylcellulose (HPMC)	P407 25 % w/v HPMC 1 % w/v	23	21	630 cP[d]	430 cP[d]	Salem et al. [55]

	Carrageenan (CGN)	P407 20 % w/w CGN 0.2 % w/w	22	22	Ca. 13000 Pa[a]	Ca. 13000[a]	Liu et al. [49]
	 Hyaluronic Acid (HA)	P407 20 % w/v HA 0.05 % w/v	27	29	10 kPa[a]	7 kPa[a]	Nascimento et al. [75]
		P407 15 % w/w P188 10 % w/w HA 2 % w/w	37	33	0.1 kPa[a]	7.3 kPa[a]	Mayol et al. [84]
	 Chitosan (CH)	P407 16 % w/w CH 1 % w/w	32	32	N/A	10 kPa[a]	Gratieri et al. [88]
	 Alginate	P407 14 % w/w AL 0.1 % w/w	Refer to article[d]				Lin et al. [90]
Nanoparticles	Chitosan nanoparticles	P407 20 % w/v CSNPs 60 mg/ 4 ml	28	27	88 Pa.s[d]	100 Pa.s[d]	Timur et al. [91]
	Poly(isobutylcyanoacrylate)	P407 20 % w/w (PIBCA) 10 mg/ml	21	21	20 kPa[a]	18 kPa[a]	Pradines et al. [94]
		P407 20 % w/w (PIBCA) 20 mg/ml	21	20	20 kPa	17 kPa[a]	
	Laponite Silicate nanoparticles	P407 20 % w/w LSN 3 % w/w	22	26	Ca 10 kPa[a]	Ca 10 kPa[a]	Wu et al [58]

a: the Storage modulus (G`); b: minimal weight required to push the apparatus 5 cm; c: softness index; d: viscosity. Note: the different values of G` and Tgel for the P407 at the same concentration is because of the varied techniques used to test the gels (stress, frequency and temperature ramp rate) . N/A indicates that the data is not present, or not extractable in an appropriate form

5. Concluding remarks

P407 has great potential in pharmaceutical formulations as a thermogelling material for topical drug delivery or injection. However, poloxamer 407 thermogel's weak mechanical strength, mucoadhesion and rapid dissolution limit its performance. Thus, modifying poloxamer 407 to improve these properties is at the forefront of current research interests and efforts. Many attempts have been made to chemically modify the structure of poloxamer to improve the resulting gel properties, often with great effectiveness. However, these modifications are costly, time-consuming, and if the polymer is intended to be used as a pharmaceutical, would require thorough toxicological study to satisfy regulatory bodies. The authors recommend that this be taken into consideration during the design of these materials, with future projects targeted at mitigating this risk and conducting more rigorous safety assessment in-line with those required for regulatory approval, such as those outlined by the International Pharmaceutical Excipients Council (IPEC).[96] Incorporating polymer additives into poloxamer solutions to form improved materials has reduced risk, especially when polymers with a history of use in pharmaceuticals are used. Some of these solutions improved the gel's mechanical properties, resistance to dissolution, as well as mucoadhesion. However, the majority of studies fail to generate P407 gels with improved strength to the standard 20 % w/w solution used, with higher concentrations of polymer additive having a detrimental effect on the ability of P407 to form a gel. Generally, the addition of polymer additives lead to an increase in Tgel at low concentrations, followed by a reduction in Tgel as the concentration was increased. Most of these studies are limited to simple binary blends over a small concentration range and any improvements seen thus far are small compared to chemical modification. The design of complex blends of multiple hydrophilic polymers with P407 may yield improvements in the material properties, and this approach would benefit from recent

advances in statistical design and machine learning tools.[97] There is a paucity of information regarding the mechanisms behind changes in rheological properties and there is a clear need to understand why complex, non-linear changes are seen in poloxamer properties upon the addition of polymer additives to P407 solutions. The authors suggest that analytical techniques which characterise nanostructure be combined with spectroscopy to elucidate intermolecular interactions to build a full understanding of these complex phenomena.

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