Water-soluble macromers based on 2-acrylamido-2-methyl-1-propanesulfonic acid sodium salt (Na-AMPS) for rapid *in situ* hydrogel film formation

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Abstract

The *in situ* formation of hydrogels has potential for number of biomedical applications but their generation via conventional polymerisation techniques has a number of limitations, such as toxicity and reaction time. The use of macromers in hydrogel formulations can help to overcome these limitations. In this work, we synthesize a new functionalized macromer formed via the copolymerization of 2-acrylamido-2-methylpropane sulfonic acid sodium salt (AMPs) and acid-functional monomers that can undergo a ring-opening reaction with allyl glycidyl ether (AGE) to generate the desired pendant vinyl macromer functionality. These macromers were characterized by ^{1}H nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FT-IR) spectroscopy and gel permeation chromatography (GPC) to provide evidence for successful macromer synthesis and subsequent polymerisation. Using a UV-initiated crosslinking approach with poly(ethylene glycol) diacrylate (PEGDA), the hydrogels were fabricated from the macromer solution, with the gelation time being reduced from 1200s to 10s when compared to hydrogel formation from regular vinyl monomers. While different acidic monomers result in distinct tensile properties, hydrogels containing 2-carboxyethyl acrylate (CEA) exhibit low strength but high elongation. In contrast, those with methacrylic acid

(MAA) demonstrate higher strength and lower elongation. Therefore, using a balanced combination of each is a logical approach to achieve a robust final hydrogel film. In summary, we have produced a new macromer possessing characteristics highly conducive to rapid hydrogel synthesis. This macromer approach holds potential for use in *in situ* hydrogel formation, where a viscous solution can be applied to the target area and subsequently hardened to its hydrogel. We envisage application primarily in the biomedical field.

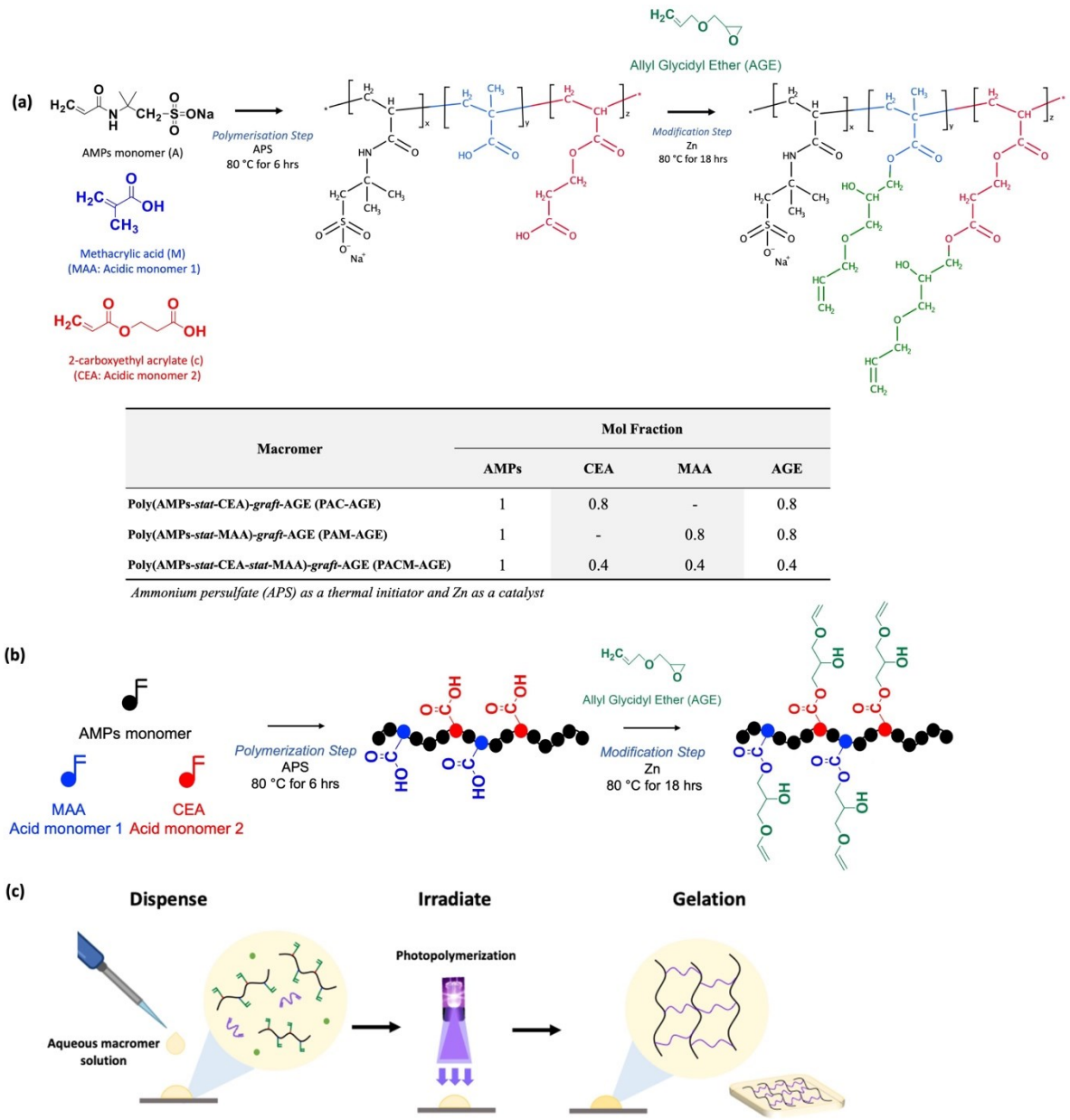
Keywords: Water-Soluble Macromer; Biomedical Hydrogel Films; 2-acrylamido-2-methylpropane sulfonic acid sodium salt; Rapid Gelation;

1. Introduction

In situ hydrogel formation involves turning a liquid precursor into a gel directly at its application site, whether on the skin or inside the body. This method, valuable for drug delivery, wound healing, and tissue engineering, fits the application site's shape and offers controlled therapeutic agent release [1-8]. Despite advances, significant challenges persist. Not only must materials be biocompatible to prevent immune reactions, but the chemicals used for gelation, such as monomers, initiators, and cross-linkers, can be harmful or allergenic. Additionally, achieving short gelation times is crucial for effective application and stability. Thus, to overcome this challenge, in-situ hydrogels are frequently created by polymerizing water-soluble telechelic polymers [9,10]. "Macromers" is a common term for telechelic polymers, yet its definition remains ambiguous. The IUPAC Compendium of Chemical Terminology (IUPAC Gold Book) suggests "macromer" might be a shortened version of "macromonomer," but it also advises against using the terms interchangeably. Macromonomers are described as oligo- or polymeric molecules with one end-group functioning as a monomer molecule [11]. Conversely, "macromer" typically denotes oligo- or polymeric molecules with one or more reactive functional groups that can either act as a monomer or form a bond with a complementary group, leading to cross-linked networks [12].

In situ hydrogels made with macromers have seen use in the field of biomedical applications. For example, Kloxin et al. innovatively employed photodegradable poly(ethylene glycol)–based hydrogels, utilizing cytocompatible macromers, which allowed for dynamic manipulation of gel properties *in situ*, influencing cell migration and differentiation [13]. Lutolf and Hubbell delved into the synthesis of hybrid hydrogels, leveraging multiarm vinyl sulfone-terminated poly(ethylene glycol) macromers, demonstrating the sensitivity of hydrogel properties to preparation states and macromer structures [14]. Park et al. explored the potential of a PEG-based hydrogel as a scaffold for chondrocyte culture, emphasizing the influence of hydrogel composition on cellular activity [15]. Wathier et al. introduced dendritic macromers for hydrogel formation, highlighting their potential in sealing clear corneal incisions in ophthalmic surgeries [16]. Recently, Altuncu et al. presented a novel approach using phosphonic acid-functionalized poly(amido amine) (PAA) macromers, which were homo- and copolymerized with 2-hydroxyethyl methacrylate (HEMA) at different ratios to obtain hydrogels with various hydrophilicities [17]. Based on the literature, most utilized macromers are derived from PEG-based systems. However, this study intends to produce macromers using 2-acrylamido-2-methylpropane sulfonic acid sodium salt (AMPS). Polymers derived from AMPS are sought after because of their intrinsic benefits. The sulfonate group found in AMPS

is similar to the glycosaminoglycan in the skin's extracellular matrix, crucial for retaining and supplying moisture to the body. This trait allows AMPS hydrogels to function like artificial proteoglycans. Additionally, studies [18-20] have shown that AMPS can speed up epithelial growth, reduce pain, and promote bioactivity in ulcerative injuries. AMPS has been incorporated into various hydrogel wound dressings and injectable hydrogels due to these properties [21,22]. For example, injectable hydrogels containing sulfonate from AMPS have been crafted using thiol-based copolymers which were combined with a 4-arm acrylamide-terminated poly(ethylene glycol) through a thiol-ene click reaction [23]. In this work we use acid containing comonomers to enable the modification step. These were 2-carboxyethyl acrylate (CEA) and methacrylic acid (MAA). MAA has been used in biomedical hydrogels for over 30 years, for example, Etaflicon A contact lenses are produced from copolymers of HEMA and MAA and have been FDA approved since 1987 [24]. Compared to MAA and acrylic acid (AA), CEA has a higher molecular weight (144 g/mol versus 86 and 72 g/mol, respectively). CEA is recognized as a minimally toxic, water-soluble monomer. It has been demonstrated to alter the rheological attributes of ceramic suspensions and reduce the adverse impacts of oxygen inhibition [25]. In this study, the molar fraction ratio of AMPS to acid-containing monomer is set at 1:0.8, a proportion similar to that utilized by Nesic et al. [26]. Their research indicated that MAA/AMPS ratios ranging from 50:50 to 25:75 yielded optimal mechanical properties. This research intends to mitigate toxicity from unpolymerized monomers in hydrogel formation, focusing on macromer technology. Compared to other methods of producing hydrogels with sulfonate groups, our approach significantly reduces exposure to toxic substances by employing macromers, which, containing similar functional groups, are potentially less toxic than low molecular weight monomers, offering a more biocompatible and less hazardous alternative. The focus of this study is with the design of functionalized macromers ideally suited for thin film hydrogels. Thus, the synthesis of AMPs-based macromer via first free radical polymerization then modification with AGE (see Scheme 1). The macromer structure was characterized by ^{1}H NMR spectroscopy, FT-IR spectroscopy, and gel permeation chromatography (GPC), while fabricated film hydrogels were characterized by gelation time, swelling ratio, contact angle, rheometry, and universal tensile testing for physical and mechanical properties.



Macromer	Mol Fraction			
	AMPs	CEA	MAA	AGE
Poly(AMPs-*stat*-CEA)-*graft*-AGE (PAC-AGE)	1	0.8	-	0.8
Poly(AMPs-*stat*-MAA)-*graft*-AGE (PAM-AGE)	1	-	0.8	0.8
Poly(AMPs-*stat*-CEA-*stat*-MAA)-*graft*-AGE (PACM-AGE)	1	0.4	0.4	0.4

Ammonium persulfate (APS) as a thermal initiator and Zn as a catalyst

Scheme 1 (a) Synthesis of macromer, PACM-AGE, *via* free-radical polymerization of 2-acrylamido-2-methylpropane sulfonic acid (AMPs) in water using two acid monomers, methacrylic acid (MAA) and 2-carboxyethyl acrylate (CEA) at 80 °C, followed by modification step of allyl glycidyl ether (AGE) at 80 °C. (b) Scheme of macromer synthesis were prepared by statistical copolymers of AMPs (black), CEA (red), and MAA (blue). The pendant carboxylic groups were modified by AGE has attached with novel allyl groups (green) in the structure. (c) Fabrication of film hydrogel by using photopolymerization from aqueous macromer solution.

2. Materials and method

2.1 Materials

2-Acrylamido-2-methyl-1-propanesulfonic acid sodium salt (AMPs) 50wt.% in H_2O, 2-carboxyethyl acrylate (CEA), methacrylic acid (MAA), ammonium persulfate (APS), allyl glycidyl ether (AGE), zinc (Zn), poly(ethylene glycol) diacrylate (PEGDA) ($M_n \sim 575$ g/mol), diphenyl(2,4,6-trimethylbenzoyl phosphine oxide) (TPO), and acetone were purchased from Sigma-Aldrich (UK).

2.2 Synthesis of AMPs-based polymer: Poly(AMPs-*stat*-CEA-*stat*-MAA) (PACM)

Initially, the polymer based on AMPs, which serves as the foundational material for macromer synthesis, was crafted using 4.0 g of 50 wt. % 2-acrylamido-2-methyl-1-propanesulfonic acid sodium salt (AMPs) in water along conjunction with two acidic monomers: 0.5044 g of 2-carboxyethyl acrylate (CEA) and 0.3012 g of methacrylic acid (MAA) (see Scheme 1). The polymerisation process was facilitated through a free radical polymerisation approach, utilising 0.04 g of ammonium persulfate (APS) as a thermal initiator, with 20 ml of water functioning as the solvent medium. This method involved heating the mixture in a water bath at 80 °C for 6 hours, with continuous stirring at 320 rpm, under ambient air conditions. The detailed compositions involved in the synthesis of these AMPs-derived polymers are shown in Table 1. This procedure culminated in the formation of a statistical copolymer, designated as Poly(AMPs-*stat*-CEA-*stat*-MAA). For comparative analysis, polymers of AMPs reacted with only CEA or MAA were also synthesized and used as control samples.

Table 1. Compositions for the synthesis of AMPs-based polymers and macromers.

Code	Synthesized Samples	Monomers (mol eq.)			Reactants (mol eq.)		
		AMPs	CEA	MAA	AGE	APS	Zn
	Copolymers						
PAC	Poly(AMPs-*stat*-CEA)	1.0	0.8	-	-	0.02	-
PAM	Poly(AMPs-*stat*-MAA)	1.0	-	0.8	-	0.02	-
PACM	Poly(AMPs-*stat*-CEA-*stat*-MAA)	1.0	0.4	0.4	-	0.02	-
	Macromers						
PAC-AGE	Poly(AMPs-*stat*-CEA)-*graft*-AGE	1.0	0.8	-	0.8	0.02	0.06
PAM-AGE	Poly(AMPs-*stat*-MAA)-*graft*-AGE	1.0	-	0.8	0.8	0.02	0.06
PACM-AGE	Poly(AMPs-*stat*-CEA-*stat*-MAA)-*graft*-AGE	1.0	0.4	0.4	0.8	0.02	0.06

2.3 Synthesis of AMPs-based macromer: Poly(AMPs-*stat*-CEA-*stat*-MAA)-*graft*-AGE (PACM-AGE)

The AMPs-based macromer was fabricated through the reaction between the poly(AMPs-*stat*-CEA-*stat*-MAA) copolymer (PACM, see Section 2.2) and 0.8 g of allyl glycidyl ether (AGE), using 0.04 g of zinc as the catalyst, which catalysed the transformation of the pendant acid groups into pendant allyl groups (Scheme 1). This reaction was keep conducted at 80 °C with water as the solvent medium, employing a stirring speed of 320 rpm sustained over a period of 18 hours, under ambient air conditions. Upon completion, the resultant macromer solution was brought down to room temperature before adding the solution dropwise into acetone, under a consistent stirring rate of 250 rpm, to foster the precipitation of the macromer. The precipitated macromer was transferred into a plastic (polyethylene terephthalate) mould, followed by a final drying phase in a hot air oven set at 60 °C for 24 hours. This procedure yielded a dried macromer ready for use. Consequently, the AMPs-derived macromer, denoted as poly(AMPs-*stat*-CEA-*stat*-MAA)-*graft*-AGE (PACM-AGE), was successfully synthesised, with its composition shown in Table 1. For comparative analysis, polymers of AMPs reacted with either CEA or MAA were also functionalised by AGE and used as control samples.

2.4 Hydrogel film fabrication using AMPs-based macromer

The hydrogel film was created using a conventional photo-initiated free radical polymerization technique, allowing for the production of hydrogel films from all starting materials utilized in this study. The AMPS based (monomer / copolymer / macromer) content in all hydrogels was kept consistent at 0.2 g. This included solid of AMPs monomer,

poly(AMPs-*stat*-CEA-*stat*-MAA) copolymer (PACM), or macromers poly(AMPs-*stat*-CEA)-*graft*-AGE (PAC-AGE), poly(AMPs-*stat*-MAA)-*graft*-AGE (PAM-AGE), and poly(AMPs-*stat*-CEA-*stat*-MAA)-*graft*-AGE (PACM-AGE). The hydrogel preparation involved mixing, 0.2 g AMPs based material, 0.1 g of PEGDA (Mn 575) as the crosslinker and 0.002 g of diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO) as the photoinitiator with 0.6 ml of DI water to serve as the solvent. This mixture was then thoroughly shaken to achieve a homogeneous macromer solution and left to stand for 24 hours at room temperature to ensure full integration of the components. Subsequently, the solution was exposed to UV radiation in air within a silicone mold, which was utilized to precisely shape the resulting hydrogels. The polymerization process to form the hydrogels was activated using a UV-LED light source, emitting at a wavelength of 395 nm with an intensity of 425 mW/cm^2.

2.5 Characterization

Chemical structures: ^{1}H NMR

Proton (^{1}H) nuclear magnetic resonance (NMR) spectra were measured in D_2O using Bruker 300 MHz UltraShield cryomagnet with Advance Neo console, with 128 scans and 256 scans on average per spectrum. Chemical shifts are expressed in ppm and internally referenced to the residual D_2O peak.

Chemical functional groups: FT-IR

Fourier transform infrared (FT-IR) spectrum were recorded on AMPs-based macromer samples, before and after modification at room temperature (16 scans accumulated per spectrum) to characterise the functional groups by using a PerkinElmer spectrum Two with UATR Two at 4000-400 cm^{-1}. All samples were kept dried at room temperature before testing.

Molecular weight: GPC

Molecular weight distributions (MWDs) were assessed by Gel Permeation Chromatography (GPC) using water (0.05% w/v NaN_3) as eluent and the flow rate was fixed at mL, 1.0 min^{-1}. Temperature of column oven and RI detector are 35 °C. The GPC set-up comprised of two columns in series which are both Agilent PL Aquagel-OH mixed-H, 7.5 x 300 mm, 8 μm, with a PL aquagel guard column in place. The molecular weight range is 6000 – 10,000,000 Da. Agilent 1260 Infinity II system with a refractive index detector. A PEG/PEO nominal M_p 106-1,500,000 Da (PL2080-0201, Agilent) was used for calibration.

Gelation Time Measurement

This critical parameter was determined by initiating the polymerization process for each sample and carefully recording the elapsed time until the formation of a solid gel was visually

confirmed. To minimize bias and variability in these visual assessments, we adopted a structured approach involving multiple independent evaluations. Specifically, five trained observers independently assessed the gelation process of each sample on separate occasions. This procedure was performed five times for each sample, ensuring an unbiased evaluation of the gelation time. The results from these observations were then averaged, and the standard deviation (± SD) was calculated, providing a robust measure of the gelation time that enhances the reliability and objectivity of our findings.

Mechanical Properties: Tensile testing and Dynamic rheology

The mechanical properties of the film hydrogels were tested on Instron 5965 at room temperature. For universal tensile tests, hydrogels with a thickness of 25 mm were cut into a dog-bone shape of Tensile strength Type 2 sample, dimension 20 mm in length and 12 mm in width (adopted from ISO 37, 2010), all samples were formed and stored at room temperature until testing. Crosshead speed was set at 10 mm min^{-1}, and at least three specimens were tested for each hydrogel sample. Also, dynamic rheological experiments were carried out using a Kinexus DSR rheometer using a cone and plate geometry with an upper plate diameter of 25 mm. During all rheological measurements, film hydrogels were divided into two states: pre-gel solution and post-fabricated gel. Aqueous macromer solution (pre-gel solution) were examined with an in-situ by using cone and plate (2° angle) to compare the quantitative evaluation of gelation process with post-fabricated gel. Fabrication of film hydrogels (post-fabricated) were achieved with an ex-situ approach by exposure to UV light at 395 nm to aqueous macromer solution (pre-gel). The hydrogels were cut to a diameter of 25 mm using a cork borer before testing. Both frequency sweep and strain sweep modes were used to examine the film hydrogels. Frequency sweep conditions: frequency range from 0.1 to 25 Hz following a logarithmic increase at 1% strain. Strain sweep conditions: strain range 0.1 – 150% with frequency 1 Hz. All measurements were conducted at 25 °C.

Contact angle (CA)

The wettability of the hydrated hydrogel surfaces was measured by contact angle (CA) using a Dataphysics Model OCA20, (Filderstadt, Germany) at room temperature. The hydrated hydrogel samples were cut in 2 cm^2 and placed on to a glass cover slide. A volume of 5 μL deionized water was dropped onto the surface sample with micrometric syringe. A static image was taken, and the contact angle was calculated using the built in OCA software. Each sample was tested 10 times (n=10) and the average values reported.

Swelling Test

The swelling behaviour of the samples was investigated by completely immersing each sample in deionised water at room temperature. Next, the swollen hydrogels were removed and weighed at selected time intervals ranging from 1 to 180 mins. Upon removal from the deionized water, the hydrogels were blotted to remove excess surface water before weighing. The swelling ratio (% swelling) was calculated based on the change in weight using the following equation (1):

$$\%\text{Swelling} = \frac{W_f - W_i}{W_i} \times 100\% \quad (1)$$

where W_i and W_f are initial weight and final weight at different times, respectively. The measurements were conducted three times for each sample and reported as the average % swelling percentage, with the standard deviation reported to indicate the level of uncertainty.

3. Results and Discussion

In this study, we successfully synthesized macromers utilizing 2-acrylamido-2-methyl-1-propanesulfonic acid sodium salt (AMPS or A) combined with the acidic monomers 2-carboxyethyl acrylate (CEA or C) and methacrylic acid (MAA or M), followed by the functionalization of their carboxylic groups using allyl glycidyl ether (AGE) - a procedure that has been explored for the first time. Our report outlines the evidence of successful product synthesis in a threefold manner: initially presenting the creation of the statistical copolymer, poly(AMPs-*stat*-CEA-*stat*-MAA) (PACM), followed by the modification of PACM using AGE, thus forming poly(AMPs-*stat*-CEA-*stat*-MAA)-*graft*-AGE (referred to as the new macromer, PACM-AGE). Lastly, the properties and formation of hydrogel films polymerized using this innovative macromer were explored.

3.1 Synthesis of PACM and its macromer, PACM-AGE

PACM and PACM-AGE were developed through a traditional free-radical polymerisation method, favoured for its simplicity, adaptability, and the extensive variety of monomers it can accommodate. The AMPs component was selected as the foundational monomer (owing to its proteoglycan-like character, as explained in the Introduction), with the integration of acidic monomers (CEA and/or MAA) to facilitate ring-opening reactions with AGE, allowing functionalisation of the macromer. This free-radical copolymerisation

procedure facilitates the amalgamation of various monomers into singular polymer chains, consequently generating copolymers. These copolymers are characterised by a statistical distribution of monomers along the polymer chain (see Scheme 1). Analytical tools such as ^{1}H NMR and FT-IR spectroscopies, and GPC were employed to verify the successful synthesis.

3.1.1 Verification of Chemical Structures by ^{1}H NMR spectroscopy

The chemical structures of PACM and the resultant PACM-AGE macromer were analysed using ^{1}H NMR spectroscopy, the results of which are depicted in Figure 1. This analysis clearly shows the anticipated changes in the proton environments of PACM before and after modification with AGE. Figure 1a details the specific chemical shifts found within the ^{1}H NMR peaks, confirming the signature peaks of PACM. Meanwhile, Figure 1b presents the chemical shifts associated with PACM-AGE, highlighting the presence of newly incorporated allyl groups originating from the AGE modification at chemical shifts of 4.0, 5.2, and 5.8 ppm. This shift in chemical structure is due to the transformation of the pendant carboxylic acid groups present in CEA and MAA, within the PACM structure, into allyl groups, following the ring-opening reaction of the epoxide groups in AGE. In order to conclusively demonstrate that these newly observed allyl peaks were not a result of unreacted AMPs monomer, comparative ^{1}H NMR analyses were conducted on both purified and non-purified samples of the macromer. These investigations unequivocally verified that the allyl groups observed were integral components of the PACM-AGE structure, as substantiated by the data presented in supporting information (see ESI, Figure S1).

Furthermore, the effective functionalisation of the macromer was evidenced by comparing the ^{1}H NMR spectra of the epoxide compound (AGE monomer) with the newly functionalised macromer (Figure 2). Notably, the disappearance of distinct epoxide proton peaks at 2.7 and 2.8 ppm (from the AGE monomer) was observed in the ^{1}H NMR spectrum of the functionalised macromer. This served as a clear indication of the successful functionalisation process. Moreover, it was observed that the allyl peaks pertinent to the AGE monomer, located at shifts of 4.0, 5.2, and 5.8 ppm within the ^{1}H NMR spectrum, remained unaltered post-functionalisation, aligning with the expected outcomes as reported elsewhere [27].

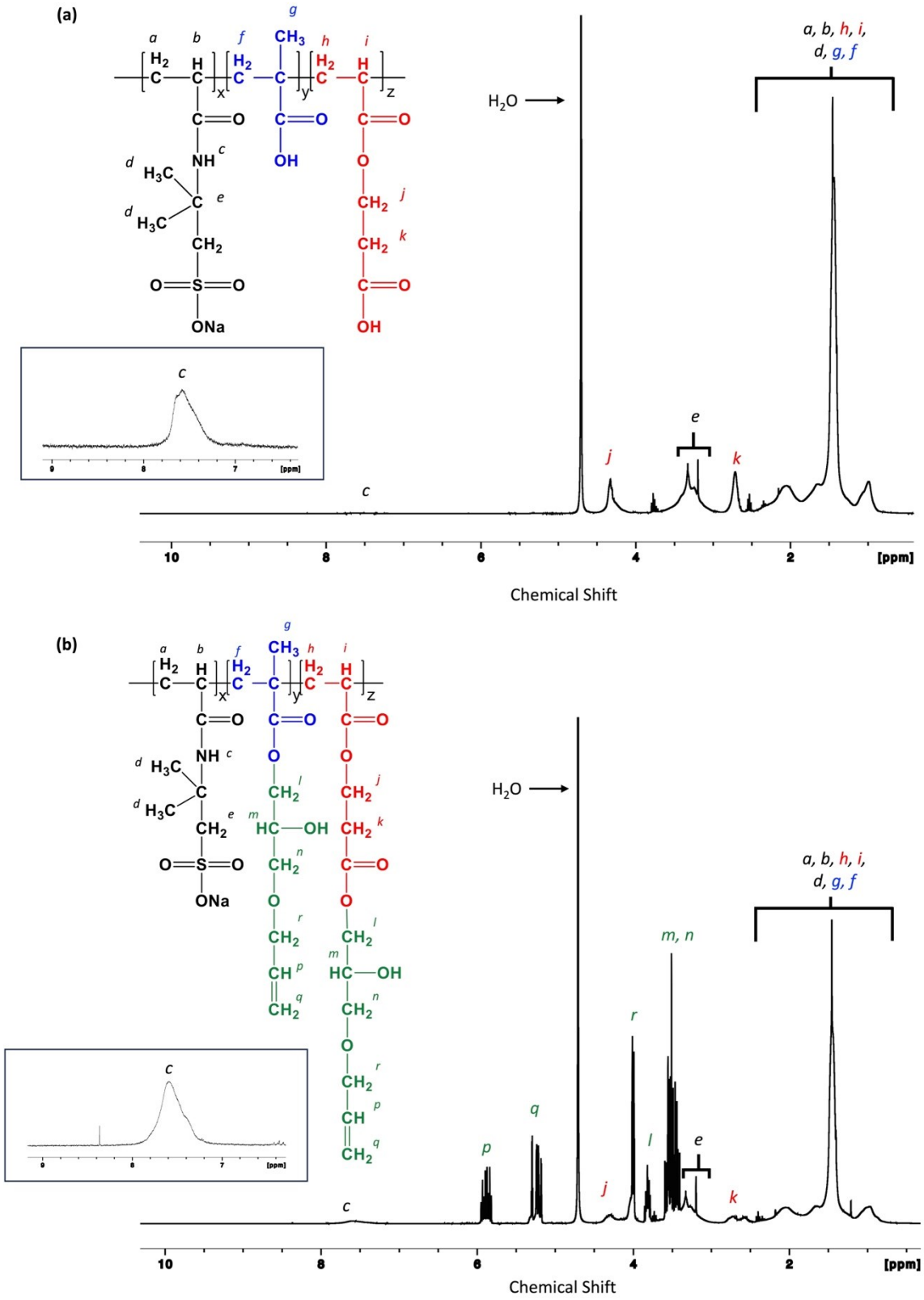


Figure 1. ^{1}H NMR spectra of (a) PACM and (b) PACM-AGE.

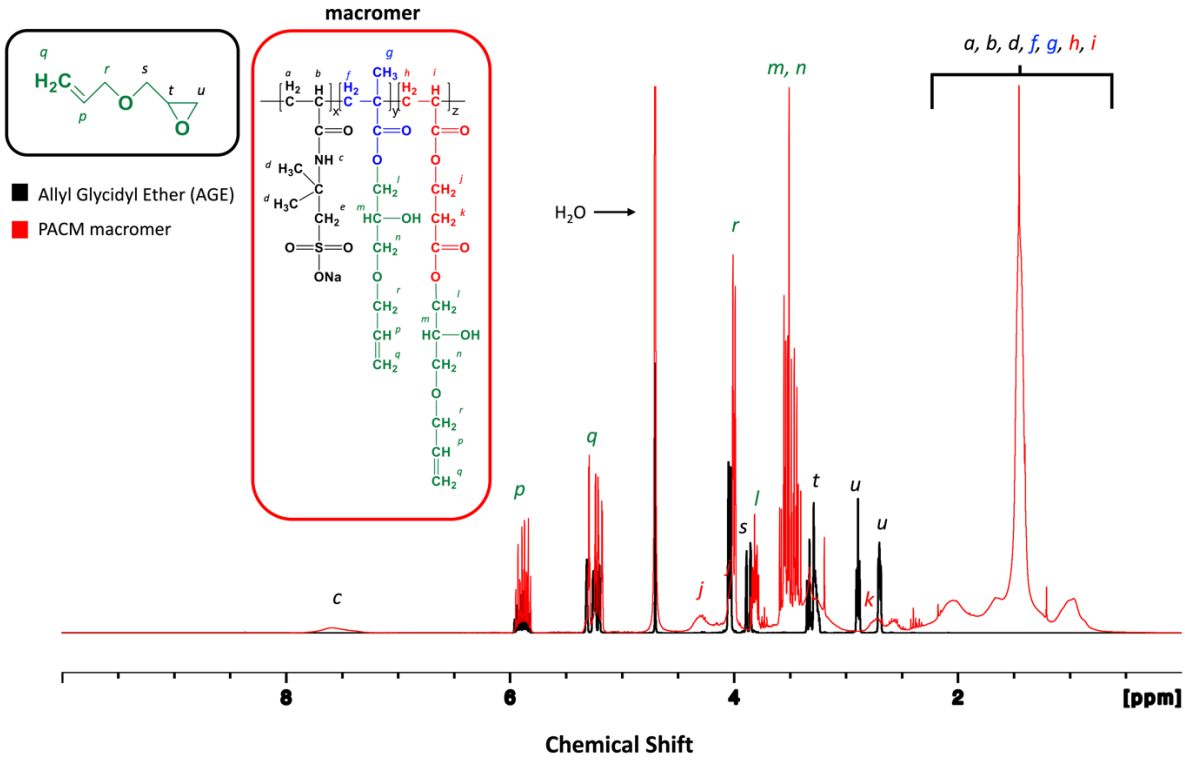


Figure 2. ^{1}H NMR spectra of PACM-AGE and AGE monomer.

^{1}H NMR spectra of poly(AMPs-*stat*-CEA) (PAC), poly(AMPs-*stat*-CEA)-*graft*-AGE (PAC-AGE), poly(AMPs-*stat*-MAA) (PAM) and poly(AMPs-*stat*-MAA)-*graft*-AGE (PAM-AGE) were also analysed to confirm the successful incorporation of allyl groups (see ESI, Figures S2 and S3). Additionally, a kinetic study indicated the successful reaction of most monomer with vinyl peaks at around at 5.6, 6.2 and 6.4 ppm disappearing after polymerization for 1h with complete disappearance after 6 hours (see ESI, Figure S4).

3.1.2 Verification of Functional Groups by FT-IR spectroscopy

FT-IR spectroscopy further corroborated the successful synthesis of the macromer. The presence of the allyl groups is evidenced by the C=C-H bending at approximately 920 cm^{-1}, a characteristic feature of AGE, as shown in Figure 3.

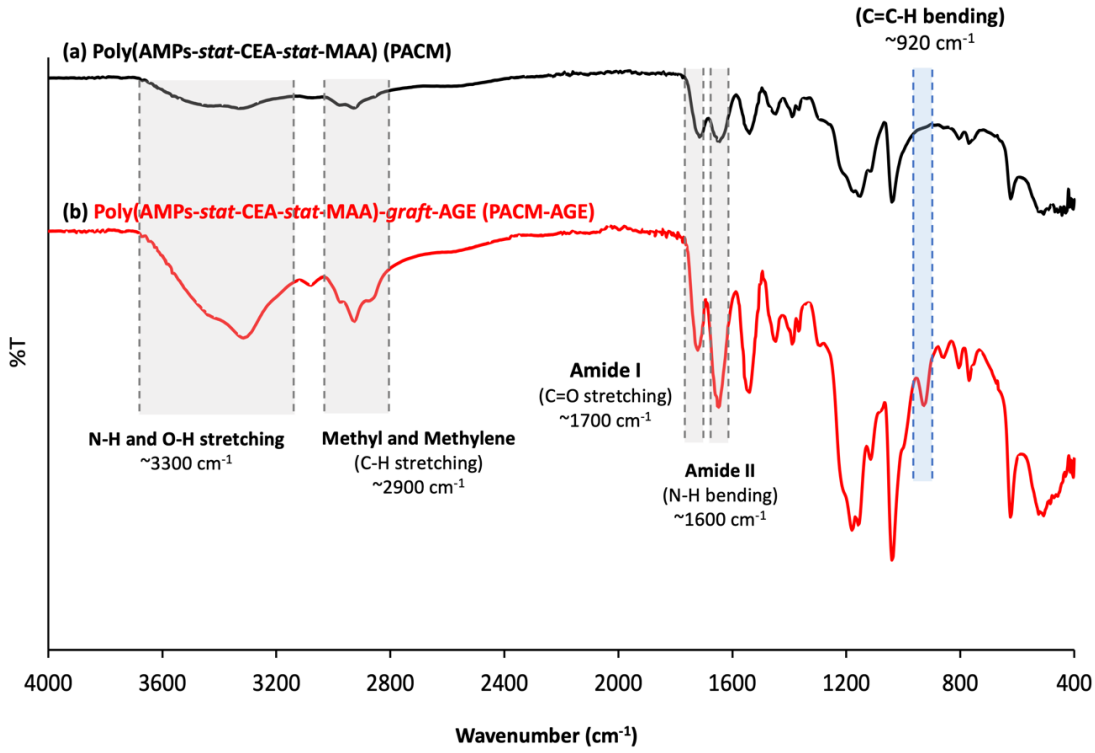


Figure 3. FT-IR spectra of (a) PACM before modification (black) and (b) PACM-AGE after modification (red).

3.1.3 Molecular Weight Assessment by Gel Permeation Chromatography

GPC was used to corroborate the successful modification of the PACM-AGE macromer by observing changes in its molecular weight profile when compared with the PACM precursor (Figure 4). As expected, a shift was observed towards higher molecular weight, with an M_n value of 103,400 g/mol for PACM ($Đ = M_w/M_n \sim 1.72$) to 136,500 g/mol ($Đ \sim 3.28$) for the PACM-AGE macromer. The noticeable increase in the dispersity ($Đ$), caused by the emergence of the high molecular weight shoulder in the GPC profile, is tentatively attributed to a small amount of branching during the AGE functionalisation step, which may be due to the unwanted side reaction of the hydroxyl group generated through the ring-opening of AGE with carboxylic acid groups on neighbouring polymer chains.

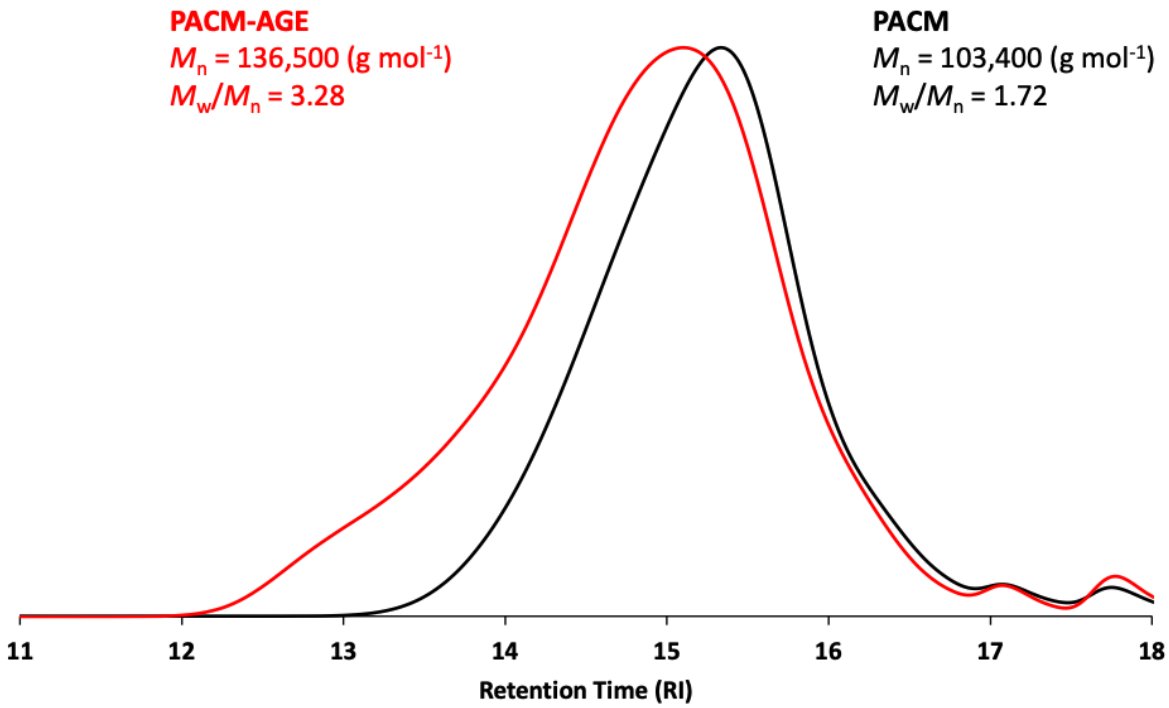


Figure 4. GPC profiles recorded for PACM and PACM-AGE.

In order to understand the system further, and potentially target different yields, molecular weights and molecular compositions (if required for future structure-property relationship studies), a brief kinetic analysis was carried out. GPC traces showed the evolution of average molecular weight of the PACM copolymers during the polymerisation, from 30 minutes to 12 hours (see ESI, Figure S5). As expected for conventional free-radical polymerisation, the average molecular weight decreased with polymerisation time in the first six hours of polymerisation.

3.2 Synthesis of copolymers (PAC and PAM) and macromers (PAC-AGE and PAM-AGE) with one additional acidic monomer

A comprehensive analysis was conducted on the synthesis of copolymers where just one additional acidic monomer was added to AMPS, poly(AMPs-*stat*-CEA) (PAC) and poly(AMPs-*stat*-MAA) (PAM), evaluating aspects such as their chemical structures, functional groups, and molecular weights. This analysis included the study of the synthesis of respective macromers, poly(AMPs-*stat*-CEA)-*graft*-AGE (PAC-AGE) and poly(AMPs-*stat*-MAA)-*graft*-AGE (PAM-AGE). Figure 5 presents the results of this analysis, alongside the data for PACM and PACM-AGE. ^{1}H NMR spectroscopy again showed new peaks in the modified copolymers (*i.e.* macromers), in line with the changes observed for PACM and PACM-AGE.

These shifts, specifically occurring at 4.0, 5.2, and 5.8 ppm, signal the successful incorporation of allyl groups. FT-IR spectroscopy revealed the presence of C=C-H bending from allyl groups at 920 cm^{-1}, further supporting the successful incorporation of AGE, and GPC traces corroborated these findings, revealing higher molecular weight in all macromers when compared to their copolymer precursors. These data confirm that the macromers were successfully made. The PAC-AGE, PAM-AGE, and PACM-AGE macromers were then used for further study, specifically for their potential to form hydrogel films following *in situ* photo-polymerisation.

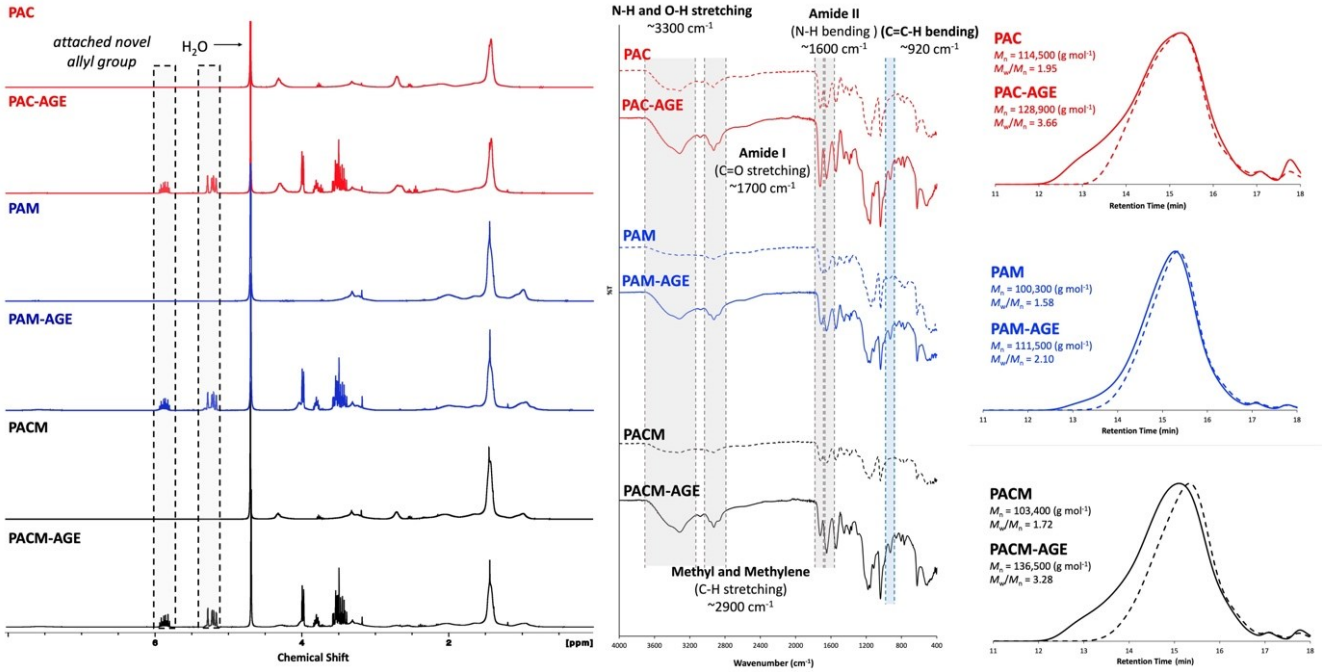


Figure 5. ^{1}H NMR, FT-IR and GPC of PAC, PAM and PACM copolymers alongside their corresponding macromers (PAC-AGE, PAM-AGE and PACM-AGE, respectively).

3.3 Rapid *in situ* polymerization to create hydrogel films

Our range of macromers (PAC-AGE, PAM-AGE, and PACM-AGE) were studied through the process of photo-polymerisation, and compared with systems based on AMPs monomer and the PACM copolymer precursor, to create durable hydrogel films. In this study, the hydrogels are composed of four principal elements: the polymer material, which can be a monomer, copolymer, or macromer; an additional crosslinking agent, specifically PEGDA (polyethylene glycol diacrylate); the photoinitiator, TPO (triphenylphosphine oxide); and water. For application of these hydrogel films as surface coatings, potentially in the biomedical industry, key properties were examined; gelation time, storage modulus (G'), and tensile strength.

3.3.1 *Gelation Time*

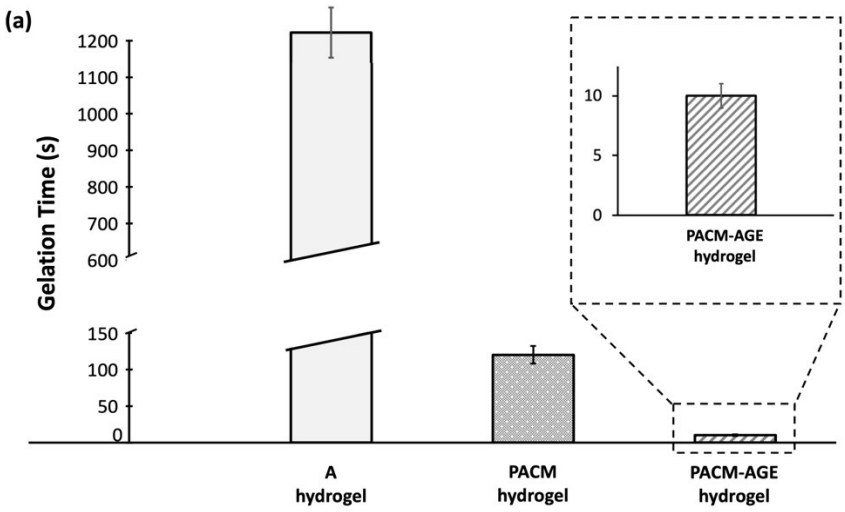
The ability of a liquid precursor to remain in place temporarily before curing is a noteworthy advantage that lends itself to various practical applications. For instance, in medical applications such as tissue engineering or wound healing, a liquid that can be precisely positioned and then solidified could provide enhanced control and conformity to complex biological structures. Visual observations were used as a tool for reporting the transition from a free-flowing viscous liquid to a self-standing hydrogel film. It was observed that the visual appearance of the polymer system changed during gelation. Initially, the liquid-like polymer formulation had a transparent and homogeneous appearance. As gelation progresses, it became more opaque with a characteristic change in clarity as time passed. These visual cues can indicate the formation of a gel network. The results of gelation time for the fabrication of hydrogel films using AMPs monomer (A hydrogel), copolymer (PACM hydrogel), and macromers (PAC-AGE hydrogel, PAM-AGE hydrogel and PACM-AGE hydrogel) are shown in Figure 6.

As presented in Figure 6a, hydrogel films synthesised using the PACM-AGE macromer demonstrated an accelerated gelation time of 10 seconds. This is significantly faster compared to films fabricated from the PACM copolymer (120 seconds) and the AMPs monomer (1200 seconds). Several different hydrogel architectures are seen in this work dependent on the system used. A standard hydrogel network is formed by the AMPS monomer combined with a PEGDA crosslinker. In contrast, the PACM copolymer (without allyl functionality) and crosslinker constitute a distinct arrangement wherein the PEG-based crosslinker constructs the framework, and the PACM copolymer interlaces or permeates throughout it, resulting in most likely a semi-interpenetrating network (semi-IPN). Meanwhile, the hydrogel created from the macromer reverts to forming a traditional hydrogel structure, with both the macromer and PEGDA forming crosslinks. These architectures are depicted in Figure S6 (see ESI, Figure S6).

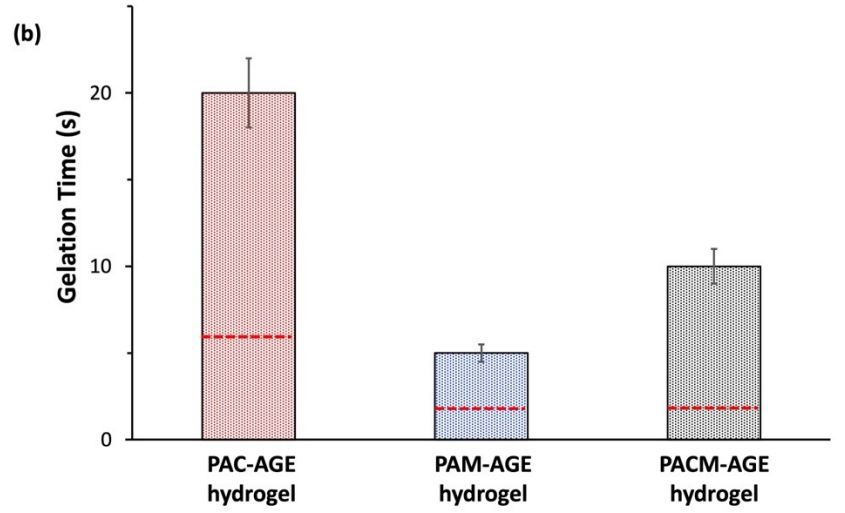
These data suggest that utilizing the macromer can expedite the gelation process by up to a factor of 10 compared to the polymer and 1000-fold when compared to the monomer. It is important to note that the compositions for all three systems are similar in terms of initiator concentration and PEGDA crosslinker content. In assessing the double bond concentration within the solution, our analysis revealed that, by incorporating PEGDA and AGE molecules, the double bond ratio between the macromer hydrogel (PACM-AGE) and the conventional hydrogel (A) is approximately 2.1:1. This calculation is based on both samples containing equal amounts of PEGDA (0.1g), with the macromer also incorporating additional double

bonds from 0.044g of AGE (under the assumption of 100% incorporation). Normalizing for these additional double bonds reveals that the macromer hydrogel achieves gelation 5 to 50 times more rapidly than its conventional counterparts (See ESI for methodology). Therefore, the principal reason for the accelerated gelation rate observed with the macromers is due to the large number of allyl groups they contain, which easily bond with PEGDA, facilitating quicker gel formation. On the other hand, the absence of such groups in the copolymer slows down the development of a fully crosslinked network, which relies on the PEGDA component only for the crosslinks (with the copolymer acting as a more passive additive that eventually forms a non-bound part of a semi-intra-penetrating network, semi-IPN). Importantly, the mixture is specifically crafted with an appropriate amount of PEGDA to encourage a rapid gelation process with suitable physical and mechanical properties. Furthermore, the prolonged gelation time observed with the monomer system can be attributed to the initial polymerisation phase of the AMPs, which occurs in conjunction with the intermolecular crosslinking in forming the polymer chain network. Therefore, the utilisation of the polymer and monomer in an aqueous medium considerably prolongs the gelation timeframe, and our findings underscore that the macromer is conducive to achieving the minimum gelation time. Furthermore, the presence of a polymeric macromer (rather than a conventional monomer) is also expected to significantly reduce the inherent toxicity or allergenicity.

An examination of hydrogel films synthesised from the range of three macromers was conducted (PAM-AGE, PAC-AGE and PACM-AGE, Figure 6b), with the dashed red lines representing the qualitative estimated juncture at which the substance transitions from a liquid state to a firm gel, as determined through visual assessments. The gelation timeframe for the hydrogel film created from PACM-AGE of 10 seconds, situating itself between the singular acidic monomer constituents of PAC-AGE (20 seconds) and PAM-AGE (5 seconds). The use of a combination of the two acidic monomers allows the tuning of the gelation time. Here, we are able to target a “goldilocks” gelation time, which is just right for the application, *i.e.* not too fast and not too slow. The system needs to gel as quickly as possible, whilst not being too fast to avoid incomplete reaction, leading to a hydrogel that has not fully reached its optimal mechanical properties and stability. Accelerated curing can cause uneven crosslinking within the hydrogel matrix, leading to areas of different mechanical strength and potentially affecting the performance of the hydrogel.



Name	Precursor (molar feed ratio)					Hydrogel Composition (g)			
	AMPs (A)	CEA (C)	MAA (M)	APS	AGE	Precursor (g)	Crosslinker PEGDA (g)	PI TPO (g)	Solvent DI water (ml)
A-hydrogel	1	-	-	-	-	0.2	0.1	0.002	0.6
PACM hydrogel	1	0.4	0.4	0.02	-	0.2	0.1	0.002	0.6
PACM-AGE hydrogel	1	0.4	0.4	0.02	0.8	0.2	0.1	0.002	0.6



Name	Macromer (molar ratio)					Hydrogel Composition (g)			
	AMPs	CEA	MAA	APS	AGE	Macromer (g)	Crosslinker PEGDA (g)	PI TPO (g)	Solvent DI water (ml)
PAC-AGE hydrogel	1	0.8	-	0.02	0.8	0.2	0.1	0.002	0.6
PAM-AGE hydrogel	1	-	0.8	0.02	0.8	0.2	0.1	0.002	0.6
PACM-AGE hydrogel	1	0.4	0.4	0.02	0.8	0.2	0.1	0.002	0.6

Figure 6. Gelation times of hydrogel films derived from: (a) monomer (AMPS), copolymer (PACM), and macromer (PACM-AGE) with PEGDA, and (b) macromers utilizing different combinations of acidic monomers. The dashed red lines denote the approximate time of the transition from a fluidic state to a stable gel, as ascertained through visual analysis.

While visual observations are subjective and may not provide precise quantitative data, the results still provide a good preliminary indication of gelation and practical information about the material performance. Thus, rheological measurements were examined to complement visual observations to obtain a more comprehensive understanding of the gelation process.

3.3.2. Rheological analysis of film hydrogels

The obtained storage modulus (G′) data for pre-gel solutions and post-fabricated hydrogels across the three formulated compositions are provided in Figure 7. Typically, in a liquid-like state, a material is devoid of a well-structured form, manifesting a comparably low storage modulus. The exertion of stress or strain prompts the material to undergo easy flow or deformation, offering negligible resistance to deformation, and consequently, low energy storage capability. The pre-gel solutions of PAC-AGE (red), PAM-AGE (blue), and PACM-AGE (grey) macromer systems exhibited storage moduli within the range of 1 to 12 Pa, while their respective post-fabricated hydrogels exhibited a range between 30,000 to 50,000 Pa. This substantial escalation in G′ from liquid-like pre-gel to hydrogel is noteworthy, which is attributed to the establishment of a three-dimensional network structure within the material. The storage modulus can also be used to calculate the crosslink density of the systems, which gives the following values. PAC-AGE Hydrogel Crosslink density (G′ = 32,310 Pa) = 4.35×10 mol^{-3}/cm^3, PAM-AGE Hydrogel Crosslink density (G′ = 49,770 Pa) = 6.70×10 mol^{-3}/cm^3, PACM-AGE Hydrogel Crosslink density (G′ = 23,190 Pa) = 3.12×10 mol^{-3}/cm^3. (See ESI, for methodology).

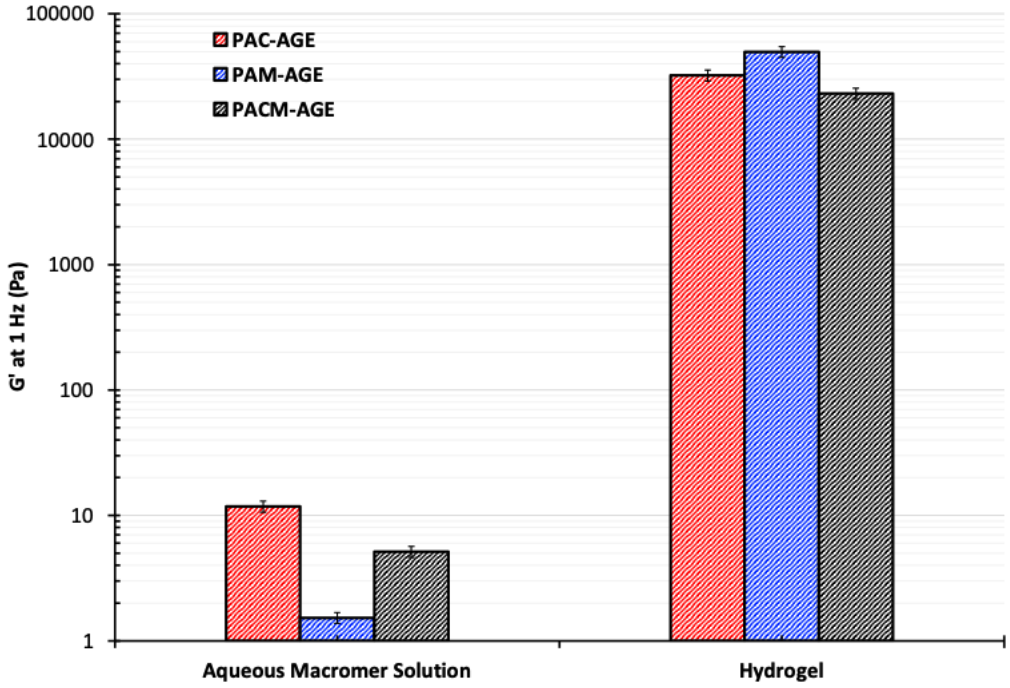


Figure 7. Storage modulus (G′) before and after exposure to UV light at 395 nm for PAC-AGE (red), PAM-AGE (blue), and PACM-AGE (grey) macromer system [G′ data recorded at angular frequency of 1 Hz].

3.3.3. Mechanical properties of the film hydrogels

The mechanical properties of the hydrogels were studied using tensile testing. Figure 8a shows the tensile stress-strain curves for AMPs-based hydrogels developed using our range of acid-functionalised macromers. The PAM-AGE-based hydrogel exhibited tensile strength and tensile strain at break point of 4.32 MPa and 33%, respectively. The shorter chains potentially contribute to a stiffer structure, predisposing the hydrogel to fractures under tensile stress. Conversely, the PAC-AGE exhibited tensile strength and tensile strain at break points of 0.30 MPa and 77%, respectively. The enhanced chain mobility of CEA facilitates augmented elasticity and elongation capabilities. This phenomenon is attributable to the heightened opportunities for molecular rearrangement and chain alignment in response to external forces. Interestingly, hydrogels crafted from a macromer that incorporates a blend of these two acidic monomers, *i.e.* PACM-AGE, displayed a tensile strength and strain at break points of 1.36 MPa and 71%, respectively. These results indicate that the tensile strength increases with an increasing proportion of MAA and diminishes with a rising concentration of CEA. It is hypothesised that an optimal balance between CEA and MAA contents can enhance the mechanical robustness of the hydrogels.

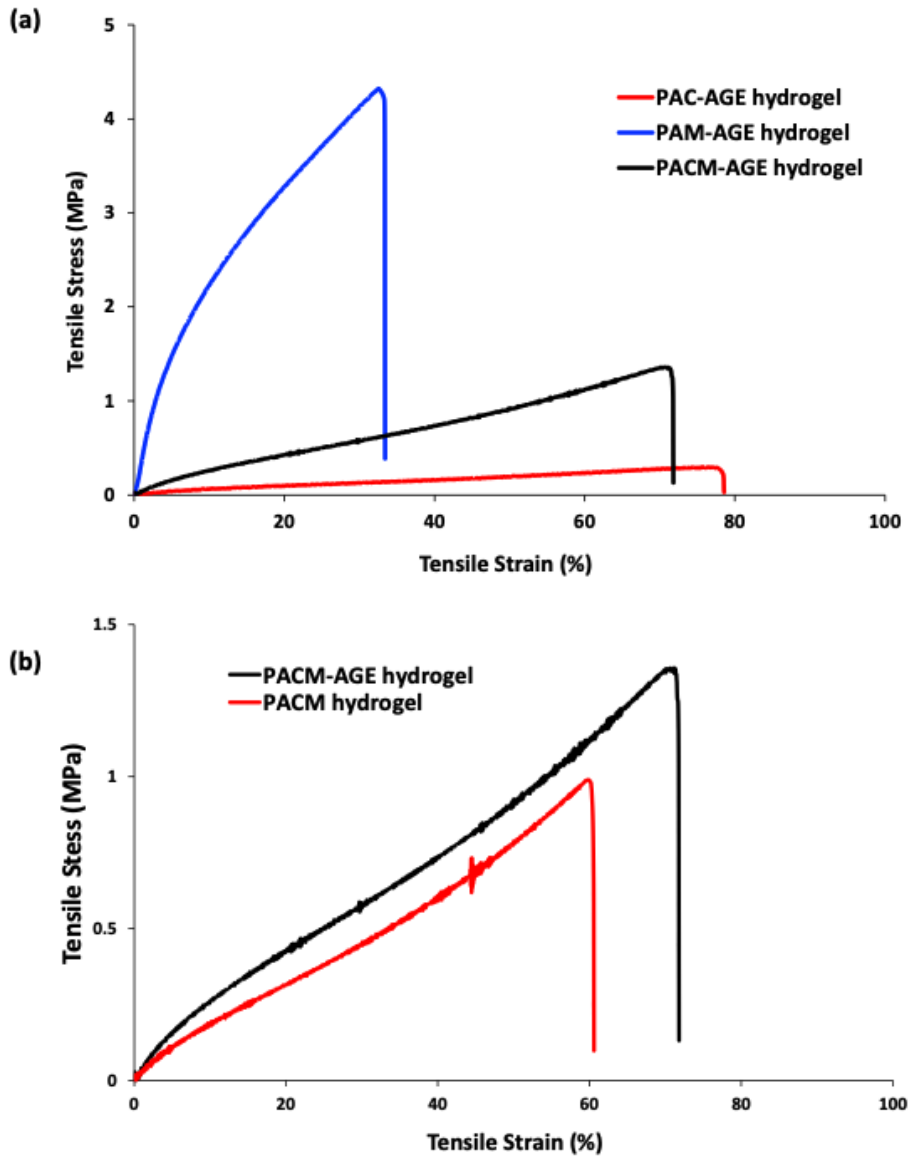


Figure 8. Stress-strain curves in hydrogel films created from macromer aqueous solutions: (a) AMPs-derived hydrogels synthesised from a range of acid-functionalised macromers, and (b) hydrogels formulated from either macromer PACM-AGE (black) or polymer PACM (red) precursors.

Figure 8b shows the stress-strain curves of hydrogels prepared from either PACM-AGE macromer (black) or PACM copolymer (red) as precursors. The tensile evaluations of the hydrogels synthesised from the PACM copolymer revealed a tensile strength and tensile strain at breaking point of 0.99 MPa and 60%, respectively. These figures were marginally lower compared to those obtained when utilizing the macromer in the fabrication process. Hydrogels derived from the macromer demonstrated a superior tensile strength over the duration of the test, along with breaking at a higher strain % (60% *versus* 77%). When comparing the

mechanical performance of AMPs/PEGDA hydrogels and PACM-AGE hydrogels tensile stress at break and elongation at break were x2 and x10 higher for PACM-AGE gels. While the AMPs hydrogels had x7 higher Young Modulus (see ESI, Figure S7).

3.3.4 Addition characterization of hydrogel films

In this section we further investigated the hydrogel films. First, we conducted a rigorous extract of a fabricated hydrogel and the checked the extract medium via NMR. The analysis revealed that less than 0.2% of the gel's total weight could be extracted over a three-hour period. Studies involving pure PEGDA gels and HEMA / PEGDA hydrogels (which included instances of PEGDA leaching at higher rates) [28] suggest that such a level of extraction is unlikely to influence the material's toxicity profile. Additionally, our preliminary toxicity studies on the macromer, which show 100% cell viability up to 250 μg/mL, using a direct method, however, this method is not suitable for higher concentrations as the method requires filtration beforehand. (See ESI, Figure S8). Next, we investigated the swelling and wettability characteristics of AMPs/PEGDA based hydrogels. Two hydrogel variants were prepared: the macromer hydrogel (PACM-AGE) and conventional hydrogel of AMPs / PEDGA (A), both utilizing identical PEGDA levels. Figure 9(a&b) show how these variants behaved when submerged in deionized water. Initial swelling was rapid within the first 15 minutes, decelerating thereafter to a plateau. The AMPs variant demonstrated a ~550% swelling ratio, markedly lower than the PACM-AGE's ~850%. Figure 9(b) shows the visual appearance before and after swelling. There is a obvious difference in appearance with the conventional hydrogel being transparent while the PACM-AGE hydrogel is opaque. The PACM-AGE hydrogel is slightly larger after swelling for 24h. The opaque appearance stems from the rapid gelation with this system. Furthermore, we explored the hydrogels' surface wettability using contact angle measurements, assessing the effects of macromer composition and macromer vs conventional AMPs / PEGDA. All gels gave contact angles corresponding the hydrophilic materials, with an increase in contact angle recorded with higher contents of CEA, suggesting decreased wettability (Figure 9(c)), this is consistence with homopolymers of CEA have been shown to have a contact angle of ~75° [25]. Figure 9(d) shows the PACM-AGE against a conventional AMPs based hydrogel, the AMPs hydrogel contains only AMPs and thus gives a lower angle (21.2° vs 58.2°).

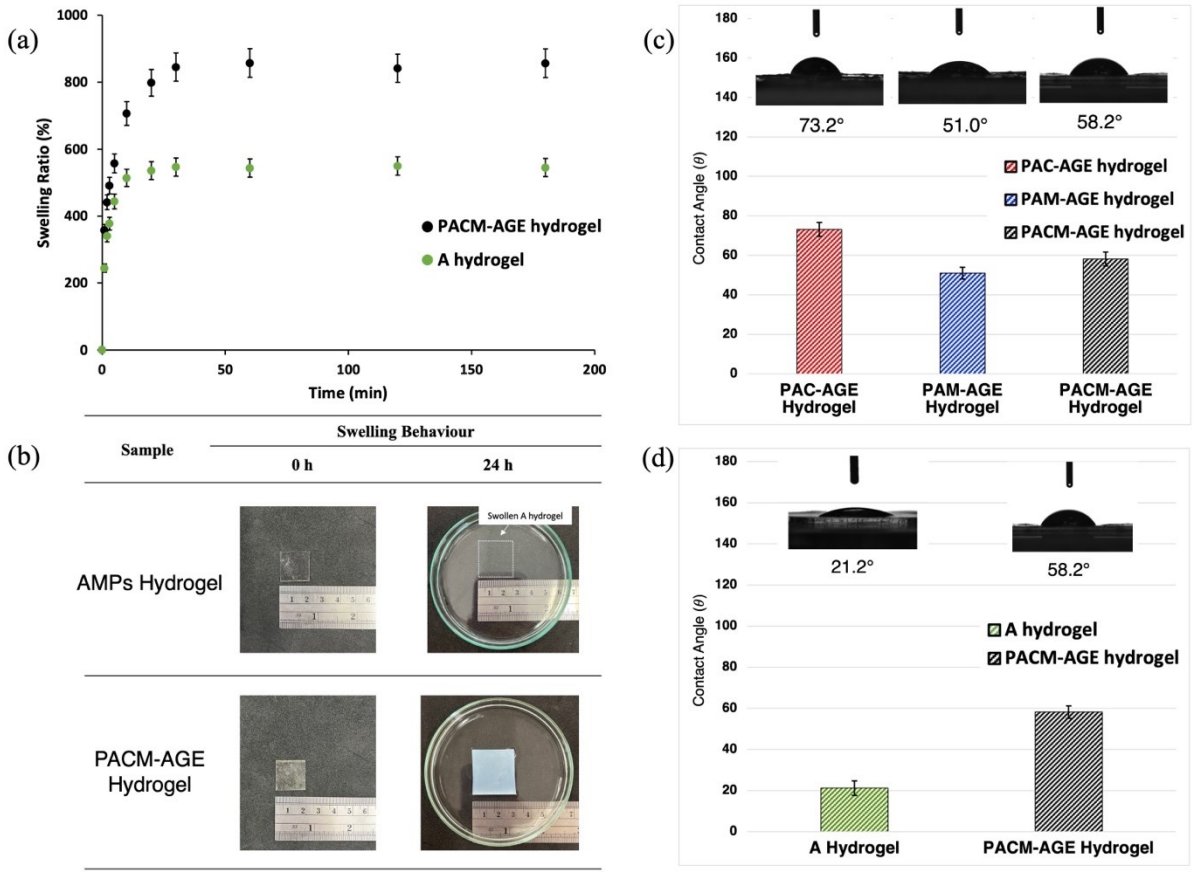


Figure 9. (a) Swelling ratio as a function of time of PACM-AGE hydrogel and AMPs hydrogel. (b) Visual appearance of the hydrogels at 0 h and 24 h; AMPs hydrogel and PACM-AGE hydrogel. (c) Water contact angle of PAC-AGE, PAM-AGE and PACM-AGE hydrogels. (d) Water contact angle of PACM-AGE hydrogels and AMPs hydrogel.

4. Conclusions

In this study, a novel macromer with multiple allyl groups was designed and synthesized for use in rapid forming hydrogels using UV-light. The primary monomer, 2-acrylamido-2-methyl-1-propanesulfonic acid sodium salt (Na-AMPS or A), was paired with acidic monomers, 2-carboxyethyl acrylate (C) and methacrylic acid (M), to equip the polymer with substituent carboxylic acid functionality to enable a ring-opening reaction with allyl glycidyl ether (AGE) to create the macromers. In total three macromers were synthesized poly(AMPs-*stat*-CEA)-*graft*-AGE (PAC-AGE) and poly(AMPs-*stat*-MAA)-*graft*-AGE (PAM-AGE) with ^{1}H-NMR and FTIR spectroscopies and GPC used to confirm the successful synthesis of the macromers. The macromers was then subsequently used to produce hydrogels films by the UV-initiated crosslinking with poly(ethylene glycol) diacrylate (PEGDA). The hydrogel properties were investigated by assessing vital parameters such as gelation time,

storage modulus (G'), and tensile strength, with additional comparison to AMPS / PEGDA gels. The successful creation of this new macromer offers a basis for further, measured exploration into its properties and potential applications. The initial findings suggest the macromer has promising attributes, including suitable mechanical properties and quick gelation time, which could be suitable for various scientific and industrial applications, including medical uses like tissue engineering or wound healing, subject to additional research.

Acknowledgements

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