Synthesis of sustainable eugenol/hydroxyethylmethacrylate-based polymers with antioxidant and antimicrobial properties†

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Received 14th September 2022 , Accepted 6th December 2022
First published on 14th December 2022

Abstract

Eugenol is a phenolic monoterpenoid, obtained mainly from clove oil and lignin, with a peculiar chemical structure containing an allyl group and a phenol group, which can be easily subjected to chemical modification. The phenol group also endows eugenol with antimicrobial activity and the ability to scavenge reactive oxygen species. Here, we present eugenol as a building block for the obtainment of antimicrobial and antioxidant bio-based polymers. So far, the approaches followed to obtain EU-based polymers involved phenol group functionalisation with polymerisable moieties, which inevitably implied the loss of the antioxidant activity of eugenol. In contrast, herein, an efficacious and low environmental impact synthetic strategy was set up to obtain an eugenol-methacrylate (EUMA) monomer bearing a free phenol group. EUMA was copolymerised with 2-hydroxyethyl methacrylate (HEMA) at different volume percentages (10%, 30% and 50%). The EUMA homopolymer was also synthesised. The obtained amorphous polymers were as thermally stable as pHEMA but more flexible (lower T_g) and hydrophobic (less swellable in water) than pHEMA. The rheological tests evidenced that all of the EU-containing polymers have good inherent elastic properties, which were retained also at high deformation frequencies (up to 80 Hz). Thanks to the presence of phenol groups in the side chain of the polymers, the pHEMA-EU copolymers showed significant radical scavenging activity and also antimicrobial activity towards a strain of *Staphylococcus epidermidis*. Overall, the synthesised eugenol-methacrylate monomer has the potential to be copolymerised with a wide number of different acrylate or vinyl monomers, thus allowing one to prepare a library of polymers with the desired physical and biological properties. The cutting-edge antioxidant and antimicrobial properties shown by the prepared copolymers open an interesting perspective towards the use of these materials in different application fields, including the food sector and biomedical field.

1. Introduction

The current environmental pressure related to phenomena like global warming and environmental pollution has led the academic and industrial communities to look for sustainable and renewable raw materials as substitutes for fossil sources. One of the main objectives of replacing oil-based molecules with bio-based ones is the production of sustainable materials, especially polymers, which may revolutionise several application sectors including the packaging, automotive and biomedical sectors. Indeed, bio-based compounds may represent “green” precursors for the synthesis of polymeric materials. This would significantly contribute to the development of the bio-economy considering that *ca*. 80% by weight of all petrochemicals are used in the polymer industry.[1]

Eugenol (EU) is a phenolic monoterpenoid obtained from different natural sources including clove oil and lignin. It is an abundant and relatively cheap raw material possessing a peculiar chemical structure, with an allyl group and a phenol group, which can be easily subjected to

chemical modification. For this reason, eugenol has been lately referred to as a promising building block for the obtainment of a broad range of bio-based polymers.[2]

The technological importance of eugenol-based polymers relies on the possibility to produce sustainable materials with cutting-edge physical and biological properties. From a physicomechanical point of view, the use of eugenol permits the inclusion of aromatic rings in the polymer backbone or side chain, which may confer chemical resistance, thermal stability, and superior mechanical properties to the polymers themselves.[3] In this context, eugenol has been proposed as a sustainable feedstock for the synthesis of bio-based alternatives of oil-based thermosetting bis-maleimide resins commonly used as matrices for multilayer printed circuit boards and advanced composite materials in the aerospace industry.[4] Also, stable latexes containing ethoxy dihydroeugenyl methacrylate and ethoxy eugenyl methacrylate have been produced and tested for adhesive applications.[5]

From a biological point of view, eugenol is known to have several pharmacological properties, such as anaesthetic, anti-inflammatory, and, of most relevance, antioxidant, anticarcinogenic and antimicrobial properties.[6] Most of the biological activities of eugenol are related to its ability to scavenge reactive oxygen species (ROS), or to prevent the generation of ROS, thanks to its phenol group. ROS are known to cause several human chronic disorders, including cancer, cardiovascular diseases and Parkinson's disease.[7]Eugenol is currently employed as an antimicrobial and anaesthetic compound in dentistry.[8] According to Globenewswire.com, the eugenol market is expected to grow steadily in the next few years especially in relation to its antimicrobial and antiviral properties.[9]The synthesis of antimicrobial polymers is one of the most hectic research sectors in materials science due to the global antimicrobial resistance issue.[10] Over the years a number of strategies has been adopted to achieve antimicrobial properties,[11–13] among which the most relevant are those relying on the use of natural compounds.[14–18]

In this framework, the synthesis of eugenol-based polymers is a quite relevant topic for the pharmaceutical field and may contribute to overcoming some issues related to the delivery of antioxidants as well as to counteracting microbial infections.[20]

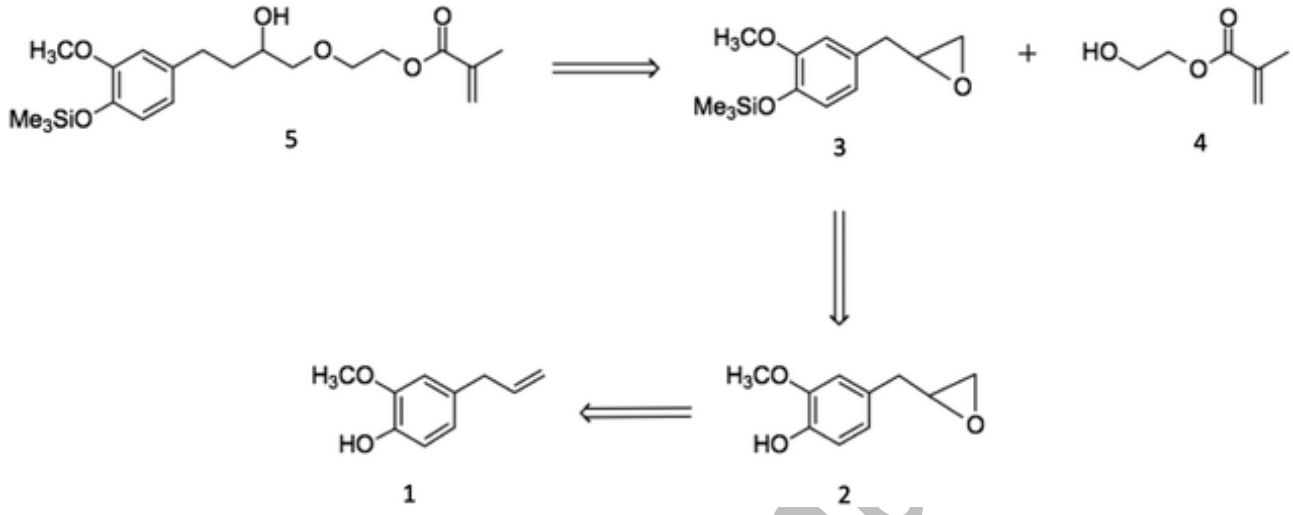
EU has several reactive groups, namely the phenol group, the allyl group and the free positions of the aromatic ring.[2] So far, for the synthesis of EU-based polymers, the eugenol phenol group has been mainly functionalized with polymerisable moieties, including methacrylate monomers,[20,21] allyl groups,[22] or ester groups.[23] Eugenol was also incorporated into benzoxazine-based phenolic resins always involving the reaction of its phenol group in this case with aromatic diamine and paraformaldehyde.[24] Although the resulting polymeric materials, in some cases, showed interesting mechanical and thermal properties, the derivatization of the eugenol phenol group inevitably implied the loss of the antioxidant activity of eugenol itself.

The contribution of the present work to the state of the art of eugenol-based polymers is the setting up of a smooth and efficacious synthetic strategy to obtain a methacrylate monomer of eugenol, *via* a new synthetic pathway, which does not involve the functionalization of the EU phenol group. Specifically, a methacrylate monomer was introduced in the EU scaffold by functionalisation of its allyl group, and prior protection of the phenol group. The obtained phenol group-bearing monomer was then copolymerised with 2-hydroxyethyl methacrylate, a biocompatible and hydrophilic monomer. The obtained polymers were characterised in terms of physical properties (swelling ability, thermal and rheological behaviour) and biological properties (antioxidant and antimicrobial activities).

2. Experimental section

2.1 Synthesis of the eugenol-methacrylate monomer

The retrosynthetic pathway for the synthesis of the eugenol-methacrylate monomer is reported in Scheme 1. Our strategy focused on the protection of the phenol group, to be deprotected in the later polymerisation step. First, the allyl group of eugenol (EU) was subjected to epoxidation to obtain compound **2**. Then, the EU phenol OH group was protected by silylation to introduce the –$SiMe_3$group and obtain compound **3**. Finally, compound **3** was reacted with 2-hydroxyethyl methacrylate (HEMA, compound **4**) to obtain compound **5**.



Scheme 1 Retrosynthetic pathway for the preparation of the eugenol-methacrylate monomer.

2.1.1 Experimental conditions to obtain compound 2. Epoxidation of the allyl group of eugenol was carried out by reacting eugenol (1 eq., 1 g, 6.1 mmol) with *m*-chloroperbenzoic acid (*m*CPBA) (4 eq., 4.2 g, 24.5 mmol). Eugenol was dissolved in dichloromethane (CH_2Cl_2, 25 mL) and *m*CPBA was added at 0 °C under a nitrogen atmosphere. After 10 minutes, the temperature was raised up to 25 °C and the reaction was carried out for 2 h. The reaction was followed by TLC (silica gel) with hexane/ethyl ether 7 : 3 as the eluent and phosphomolybdic acid in ethanol solution (10% w/v) plus a UV lamp as the detection system. After 2 h, TLC analysis evidenced the total disappearance of the starting material, so the obtained orangish mixture was neutralized with Na_2CO_3 and extracted with a separatory funnel. The organic mixture was finally dried over anhydrous Na_2SO_4 and the solvent was evaporated under vacuum. Compound **2** was used without further purification. ^{1}H-NMR spectroscopy confirmed the obtainment of the product. ^{1}H-NMR (400 MHz, $CDCl_3$) = 6.8 (d, 1H, Ar–H), 6.76 (s, 1H, Ar–H), 6.73 (d, 1H, Ar–H), 5.6 (bs, 1H, OH), 3.8 (s, 3H, –OCH_3), 3.4 (m, 1H, –CH_2CH-O-CH_2), 2.7 (m, 2H, –CH_2CH-O-CH_2), 2.6 (m, 2H, –CH_2CH-O-CH_2).

2.1.2 Experimental conditions to obtain compound 3. The –OH group of compound **2** was protected with –$SiMe_3$. Compound **2**(230 mg, 2.13 mmol, 2 eq.) was reacted with hexamethyldisilazane, HMDS (200 mg, 1.2 mmol, 1 eq.), at 150 °C for 4 h, under a nitrogen atmosphere. The reaction was followed by TLC (silica gel) with hexane/ethyl ether 9 : 1 as the eluent and phosphomolybdic acid in ethanol solution (10% w/v), molecular iodine (I_2) plus a UV lamp as the detection system. When TLC evidenced the total disappearance of compound **2**, HMDS was removed by evaporation under vacuum. Compound **3** was used without further purification. ^{1}H-NMR spectroscopy confirmed the obtainment of the product.
^{1}H-NMR (400 MHz, $CDCl_3$) = 6.8–6.7 (m, 3H, Ar–H), 3.8 (s, 3H, –OCH_3), 3.4 (m, 1H, –CH_2CH-O-CH_2), 2.8 (m, 2H, –CH_2CH-O-CH_2), 2.6 (m, 2H, –CH_2CH-O-CH_2), 0.25 (s, 9H, –Si-$(CH_3)_3$.

2.1.3 Experimental conditions to obtain compound 5. To obtain compound **5**, the epoxy group of compound **3** was opened by the OH group of HEMA. HEMA (310 mg, 2.4 mmol, 3 eq.)

was first reacted with triethylamine, TEA (242 mg, 2.4 mmol, 3 eq.), at 70 °C for 2 h under stirring. Then, compound **3** (1 equiv., 0.178 mmol) was added to the mixture and the reaction was carried out for 3 h. The reaction was followed by TLC (silica gel) with hexane/ethyl ether 6 : 4 as the eluent and phosphomolybdic acid in ethanol solution (10% w/v), molecular iodine (I_2) plus a UV lamp as the detection system. When the starting material was consumed, as shown by TLC analysis, the obtained brownish mixture was neutralized with HCl (0.05 M), extracted with CH_2Cl_2, and dried over anhydrous $Na_2SO_4$and the solvent was removed under vacuum. Compound **5** was named eugenol methacrylate (EUMA) and characterized by ^{1}H-NMR and ^{13}C NMR spectroscopy, by using tetramethylsilane as the internal reference. The sample was dissolved in deuterated chloroform ($CDCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.9–6.6 (m, ArEU), 6.1 (s, $=CH_2$), 5.6 (s, $=CH_2$), 4.2 (t, O**CH$_2$**CH_2O), 3.9 (t, OCH_2CH_2O), 3.8 (O**CH$_3$**), 3.1–3.0 (m, CH), 2.9–2.4 (m, Ar**CH$_2$** and CH_2), 1.9 (s, $=CH_3$), 0.11 (m, $Si(CH_3)_3$). The compound (10 ng mL^{-1}) in acetonitrile : methanol (2 : 1, v/v) was injected into a PE-Sciex API-3000® triple quadrupole mass spectrometer (PerkinElmer Sciex Toronto, Canada). The electrospray ionization source was operated in the positive ionization mode with a capillary voltage of 4500 V.

2.2 Synthesis of poly-2-hydroxyethyl methacrylate and HEMA–EUMA copolymers

The homopolymer poly-2-hydroxyethyl methacrylate (pHEMA) was obtained by free-radical polymerisation photoinitiated by using IRGACURE D-2959 (2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone) as the photosensitive initiator. It is known that the physical properties of pHEMA samples are largely affected by polymerisation conditions, including the presence of a chain transfer agent, which controls the polymer molecular weight and cross-linking degree.[25] To investigate such aspects, HEMA was polymerised either with or without a chain transfer agent (T), sodium metabisulfite ($Na_2S_2O_5$), which was added in two molar ratios with respect to the monomer ([T]/[M] = 0.1 and 0.5).

Specifically, the initiator (6.42 mg, 2% w/w with respect to the monomer) was dissolved in 20 µL of water and added together with HEMA (300 µL, 2.47 mmol) into a circular mold (d = 2.5 cm). When needed, the chain transfer agent was added in this phase (47.12 mg for [T]/[M] = 0.1 and 235.6 mg for [T]/[M] = 0.5). The polymerisation was carried out under a UV lamp for 30 min, 10 min under stirring and 20 min without stirring. The obtained polymers were washed with water to eliminate the unreacted monomer and dried in a vacuum oven.

The HEMA–EUMA copolymers were obtained by the same experimental procedure used for pHEMA by employing 2% w/w IRGACURE and a [T]/[M] ratio of 0.1, which provided a pHEMA sample with the best properties. Several EUMA/HEMA volume percentages were employed during the synthesis: 10%, 30%, 50%, and 100% v/v. The resulting copolymers were called pHEMA-EU_{10}, pHEMA-EU_{30} and pHEMA-EU_{50}, where the subscript indicates the theoretical EUMA content. The EUMA homopolymer was called pEU. At the end of polymerisation, copolymers were washed with water to eliminate the unreacted monomer and with 0.5 M HCl to remove the –$SiMe_3$group and restore the initial eugenol –OH group and dried under vacuum. The efficacy of the purification procedure was followed by UV-spectroscopy by checking the disappearance of absorbance at 350 nm.

2.3 Polymer characterization

The molecular weight of pHEMA was determined by capillary viscosimetry, by using an automatic system SCHOTT GERÄTE Ubbelohde capillary viscosimeter equipped with a SCHOTT AVS 350 ViscoSystem and a LAUDA CD15 thermostatic bath. Measurements were performed at 30 °C, in the 0.01–0.26 g mL^{-1} polymer concentration range and using dimethyl formamide (DMF) as the solvent. Under these conditions, the Mark–Houwink equation constants, K and a, were considered to be K = 10.6 × 10^{-3} mL g^{-1} and a = 0.70.[26] All of the

solutions were stirred for 1 h and filtered with GF/D Whatman® microfiber glass filter before analysis.

Fourier-transform infrared spectroscopy (FTIR) was performed in attenuated total reflection (ATR) mode using a Nicolet 6700 (Thermo Fisher Scientific, USA) equipped with a Golden Gate ATR accessory (angle of incidence 45°), at a resolution of 4 cm^{-1} and co-adding 200 scans.

Polymer thermal analysis was performed by both thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA was carried out employing a Mettler TG 50 thermobalance (Mettler Toledo, Columbus, OH, USA), under N_2 flow, in the temperature range of 25–500 °C, at a 10 °C min^{-1} heating rate. DSC was performed using a Mettler TA-3000 DSC apparatus, at 10 °C min^{-1} in the −150–120 °C temperature range, under N_2 flux.

The swelling ability (SA) of polymers in water was determined by the immersion of weighed samples (W_0) in water at room temperature, by following the ISO62 standard method (Plastics—Determination of water absorption). At determined times, the sample was collected, lightly dabbed on filter paper to remove excess solvent and weighed (W_t). The test was carried out until saturation. Water uptake was defined as follows:

$$SA(\%) = \frac{W_t - W_0}{W_0} \times 100$$

Three parallel swelling experiments were performed for each sample and the data were reported as average value ± standard deviation.

The rheological behaviour of polymers was studied using a rotational rheometer with plate–plate geometry on circular samples (25 mm in diameter and 1 mm in thickness) at 37 °C. Amplitude sweep tests were performed in the strain-control mode for the strain amplitude in the range of 0.1–2%, the oscillation frequency being 1 Hz. Frequency sweep tests were performed in the stress control mode. The stress value was selected so that the target strain (0.5%) was within the limits of linear viscoelasticity as determined from the amplitude sweep studies. The frequency range was typically between 0.1 and 100 Hz.

2.4 Evaluation of the antioxidant activity of polymers

Evaluation of the antioxidant activity of polymers was carried out by using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical method.[27] DPPH is a stable radical that turns from violet to yellow when reduced to DPPH-H by the reaction with an antioxidant. Therefore, by following the absorbance decrease at 520 nm by UV-vis spectroscopy, it is possible to quantify the scavenging activity of the tested antioxidant compound.

First of all, the ability of eugenol to scavenge DPPH was evaluated. A solution of DPPH (1.7 mg) in methanol (20 mL, 1.5×10^{-4} M) and a solution of EU (4 mg) in methanol (10 mL) were prepared. Then, 2 mL of DPPH solution and various volumes of the EU solution (10, 30, 50, and 100 µL) were added into a UV cuvette to obtain solutions with different EU concentrations. Methanol was finally added up to 4 mL for all the solutions.

After 30 min, the absorbance of the solutions was measured at 516 nm and the radical scavenging activity (RSA) was determined as follows:

$$RSA(\%) = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

where A_0 is the absorbance value of the DPPH solution without antioxidant and A is absorbance of the solution after reaction with the antioxidant. By plotting the amount of residual DPPH, evaluated from a calibration curve, as a function of compound concentration, the effective

concentration (EC_{50}), defined as the amount of compound needed for decreasing the initial DPPH concentration by 50%, was determined.

As far as the determination of the antioxidant activity of polymers is concerned, the same method was used but with some modifications due to the insolubility of the polymers in methanol.[28] Specifically, different polymer amounts (5, 10 and 15 mg) were suspended in the DPPH solution and, after 30 min, the supernatant was subjected to UV-vis spectroscopy for the determination of the residual DPPH amount and the radical scavenging activity.

2.5 Antimicrobial activity

The antibacterial activity of polymer samples was assessed against a standard strain of *Staphylococcus epidermidis* (ATCC 35984), known to be an opportunistic pathogen often involved in nosocomial infections.

Briefly, a bacterial inoculum at 1×10^8 CFU per mL in Muller–Hinton broth (M–H) with an optical density of 0.125 at 625 nm was first prepared. Then, 200 µL of the bacterial inoculum was added into the wells of a 96-well culture plate containing different amounts of polymers (2, 4 and 8 mg) and 1.8 mL of MH broth. The negative control was the MH broth with no bacteria while the positive control was the MH broth with the bacterial inoculum without the polymer. Following the incubation of plates at 37 °C overnight, the optical density (OD) of the solutions was measured at 625 nm to determine the bacterial growth percentage (BG, %):[29]

$$BG(\%) = \frac{OD_{sample} - OD_0}{OD_{positive\ control} - OD_0} \times 100.$$

2.6 Statistics

Analysis of variance comparisons were performed using Mini-Tab. Differences were considered significant for $p < 0.05$. Data are reported as mean ± SD.

3. Results and discussion

Eugenol-based polymers are a family of materials with incredibly versatile physical and biological features holding promises for different applications, including the food packaging and the biomedical field. With the aim of producing antioxidant eugenol-based polymers, a novel synthetic strategy with low environmental impact was set up to obtain a methacrylate monomer of eugenol possessing a free phenol group, known to confer several biological activities on the compound.

The success of the three-step procedure setup to obtain EUMA was confirmed by ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectra of the intermediate products are reported in the ESI (ESI, Fig. S1–S3†) while the ^{1}H-NMR spectrum of EUMA (compound **5**) is reported in Fig. 1. The ^{1}H-NMR spectrum of EUMA suggests the presence of a mixture of compounds (HEMA and EUMA). The signals of EUMA are in the 6.9–6.6 ppm spectral range (aromatic protons), at 3.8 ppm (OCH_3), at 2.9–2.4 ppm ($ArCH_2$ and CH_2), and at 3.1–3.0 ppm (CH). Instead, the signals at 6.1–5.6 ppm ($=CH_2$) and 1.9 ppm (CH_3) belong to both HEMA and EUMA. Similarly, the ^{13}C-NMR spectrum of EUMA (ESI, Fig. S4†) shows the presence of signals of both EUMA and HEMA products. Liquid chromatography mass spectrometry (ESI Fig. S5†) confirmed the obtainment of the product with an exact mass of 383. The product was used for polymer synthesis with no further purification step, since HEMA is a monomer added to the polymerisation procedure.

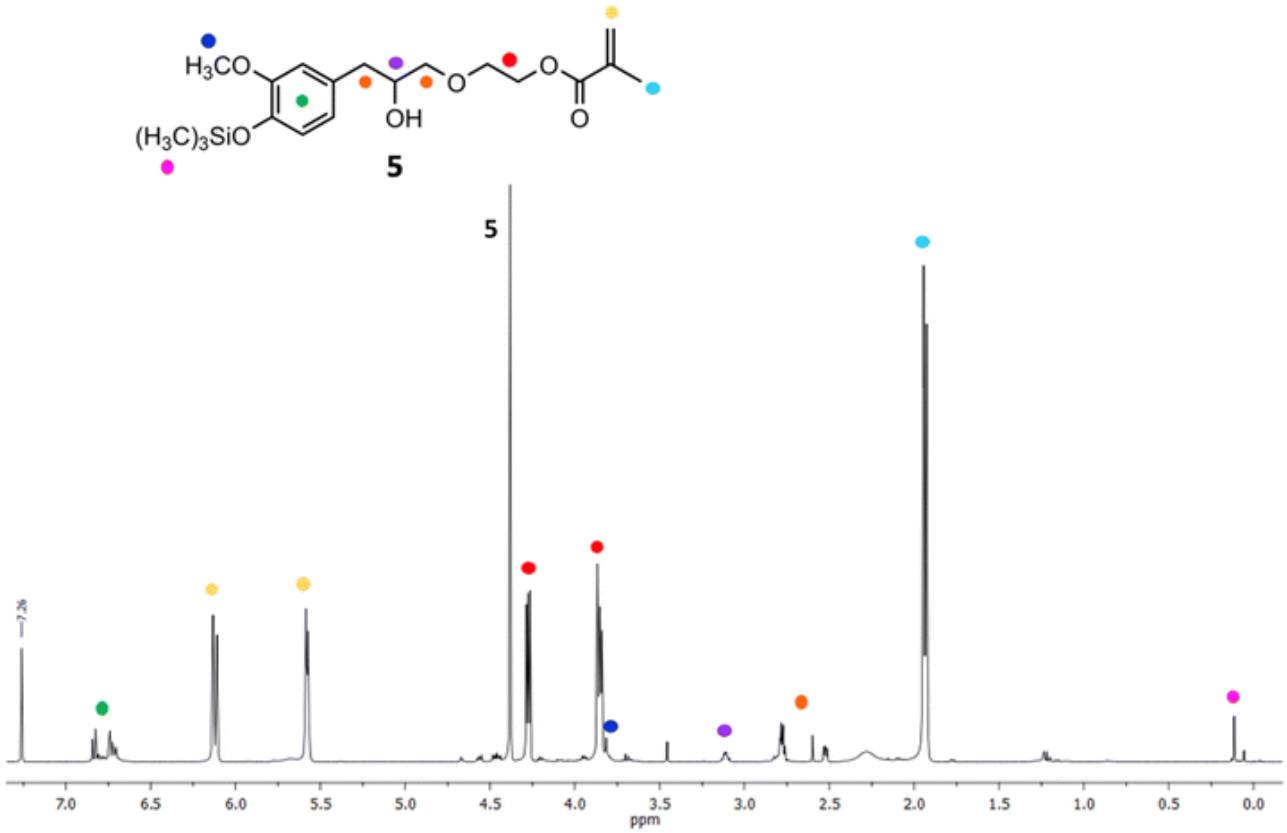


Fig. 1 ^{1}H-NMR spectra of EUMA in $CDCl_3$ (compound **5** of Scheme 1).

3.1 HEMA polymerisation

HEMA was polymerised in mass by using IRGACURE D-2959 as the photosensitive initiator in the presence or absence of a chain transfer agent. After 20 min of polymerisation, gelation of the polymerisation solution was observed suggesting satisfying polymerisation of the monomer as confirmed by FTIR spectroscopy where the peak at *ca*. 1640 cm^{-1} related to the HEMA C=C stretching almost disappeared (Fig. 2).

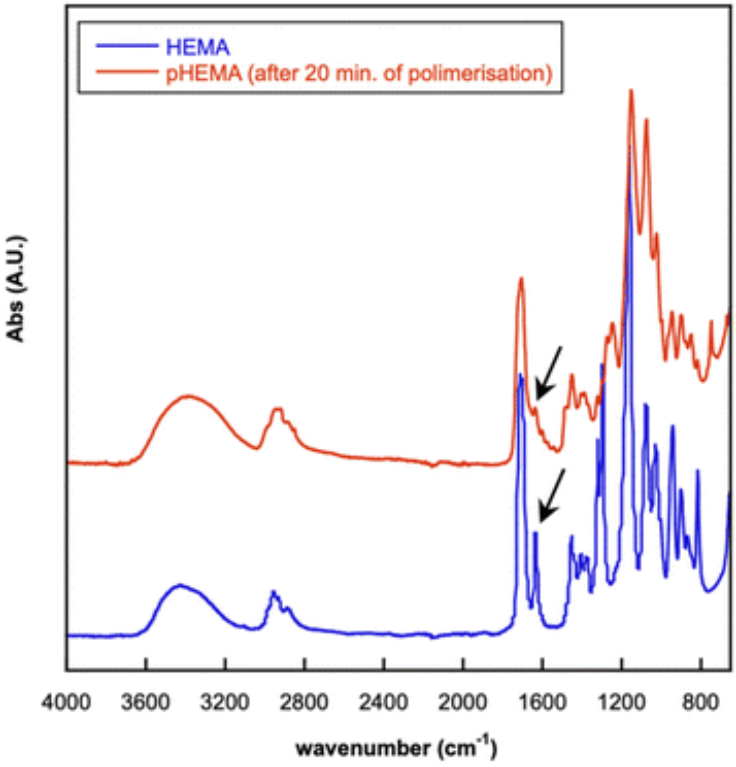


Fig. 2 FTIR spectra of HEMA and pHEMA obtained with Irgacure (2%) and [T]/[M] ratio of 0.1, after 20 min c
indicate the HEMA peak decreasing after polymerisation.

The pHEMA sample obtained without chain transfer was insoluble in water and in common organic solvents presumably because of inter and intramolecular crosslinking reactions.[25] In contrast, the two polymer samples obtained in the presence of the chain transfer agent ([T]/[M] = 0.1 and 0.5) were soluble in DMF.

Therefore, viscosimetric characterisation was performed only on these latter samples. As expected, the increase in the chain transfer concentration, dramatically decreased the polymer molecular weight. Indeed, the viscosity average molecular weight of pHEMA was found to be 6.5×10^4 for [T]/[M] = 0.1 and 400 for [T]/[M] = 0.5. Due to the very low molecular weight obtained with a [T]/[M] ratio of 0.5, further HEMA and EUMA copolymerisation was carried out with a [T]/[M] ratio of 0.1.

3.2 Synthesis of HEMA–EUMA copolymers

Three HEMA–EUMA copolymers were synthesised by photopolymerization *in situ* at varying EUMA/HEMA volume ratios (10%, 30% and 50%). The homopolymer of EUMA was also synthesized. The obtained polymers were found to be insoluble in water and in common organic solvents. We can hypothesise that the insolubility of the polymers is a consequence of the performed *in situ* bulk photo-polymerisation. Indeed, this procedure can produce high molecular weight polymers, which are prone to physical crosslinking and gelation by H-bond interactions. The presence of eugenol in the sidechain can further promote polymer physical crosslinking by π–π stacking interactions of the aromatic moieties. They showed a yellowish-brown colour (Fig. 3), related to the presence of eugenol that is a yellow liquid. FTIR-ATR spectroscopy confirmed the copolymerisation of the two monomers. In Fig. 4, the FTIR spectra of the different polymers are reported in the 2000–650 cm^{-1} spectral range. As can be seen, the absorption peaks related to HEMA (C═O stretching at 1720 cm^{-1}) and EU (aromatic C═C stretching at 1600 cm^{-1}) are both present.

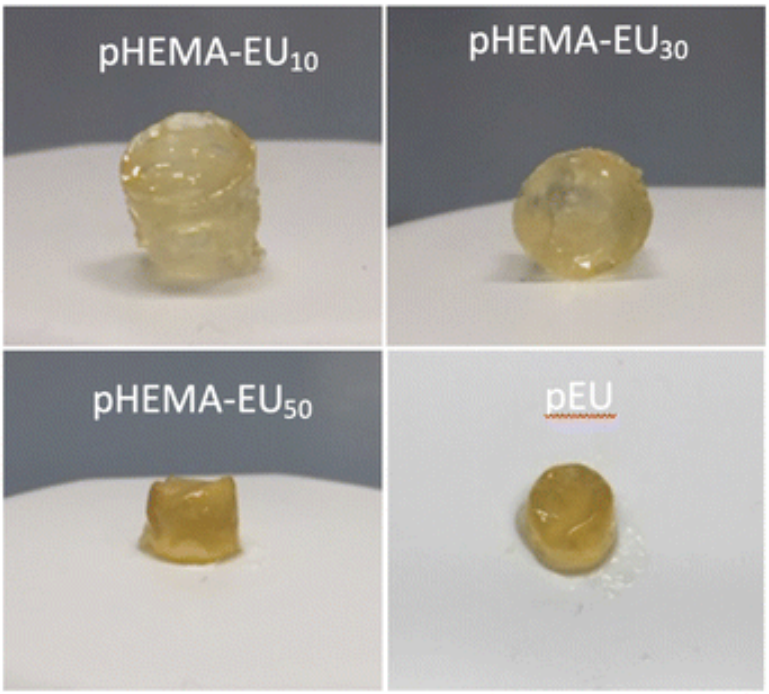


Fig. 3 Visual appearance of pHEMA–EUMA polymers.

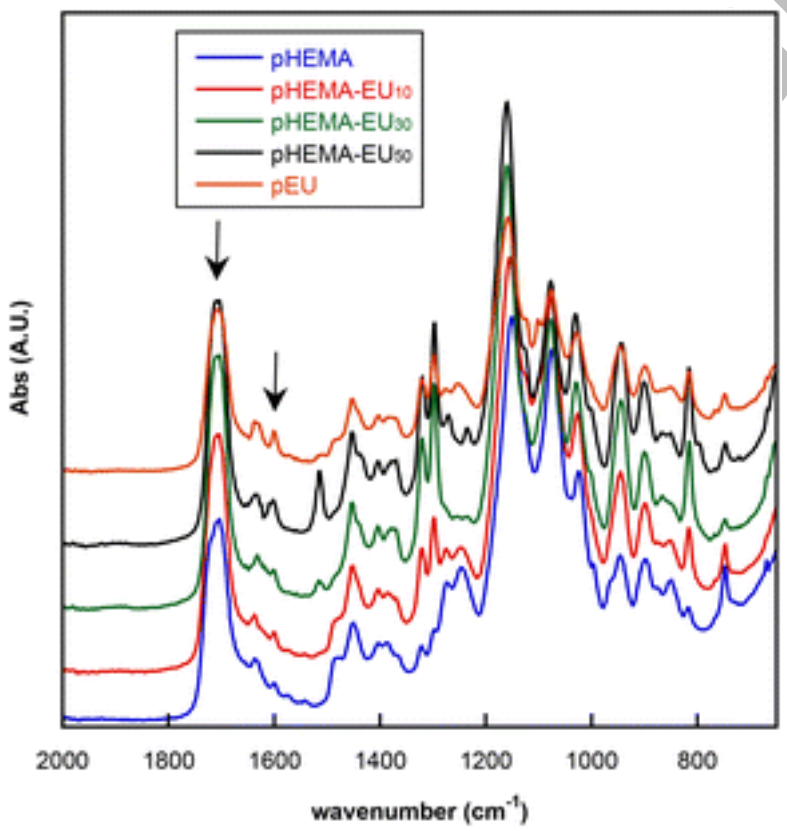


Fig. 4 Comparison of the FTIR spectra of pHEMA, pHEMA-EU$_{10}$, pHEMA-EU$_{30}$, pHEMA-EU$_{50}$ and pEU in the range.

From the ratio of the intensities of the peaks at 1600 cm^{-1} (aromatic C=C stretching of EUMA) and at 1720 cm^{-1} (C=O stretching of HEMA), the EUMA content in the copolymers was estimated (Table 1). Interestingly, an increase in the *I*(C=C)/*I*(C=O) ratio was observed with the

increase in the EUMA content in the polymerisation feed, suggesting an increasing inclusion of the EUMA in the copolymers.

Table 1 Properties of the pHEMA–EUMA copolymers and pEU. Ratio between the FTIR intensities of the C=C and C=O stretching peaks ($I_{(C=C)}/I_{(C=O)}$); glass transition temperature (T_g); swelling degree in water at the equilibrium (%). NA = not applicable NP = not performed

Sample	FTIR $I_{(C=C)}/I_{(C=O)}$	T_g(°C)	Swelling degree (%)
pHEMA	NA	50	320
pHEMA-EU$_{10}$	0.037	−77	168
pHEMA-EU$_{30}$	0.164	−80	103
pHEMA-EU$_{50}$	0.185	−85	72
pEU	0.290	−90	NP

The successful obtainment of pHEMA–EUMA copolymers by photopolymerization *in situ* opens an interesting perspective for their use in additive manufacturing for the obtainment of insoluble products with the desired geometry, topography and chemical features.

3.3 Thermal analysis

The thermogravimetric analysis carried out on EU, HEMA, and EUMA compounds evidenced that the functionalized EUMA degrades at lower temperatures (208 °C) than the starting compounds HEMA (228 °C) and EU (265 °C), even if it reaches complete degradation at higher temperatures (Fig. 5A). Presumably, derivatization affects interactions among molecules.

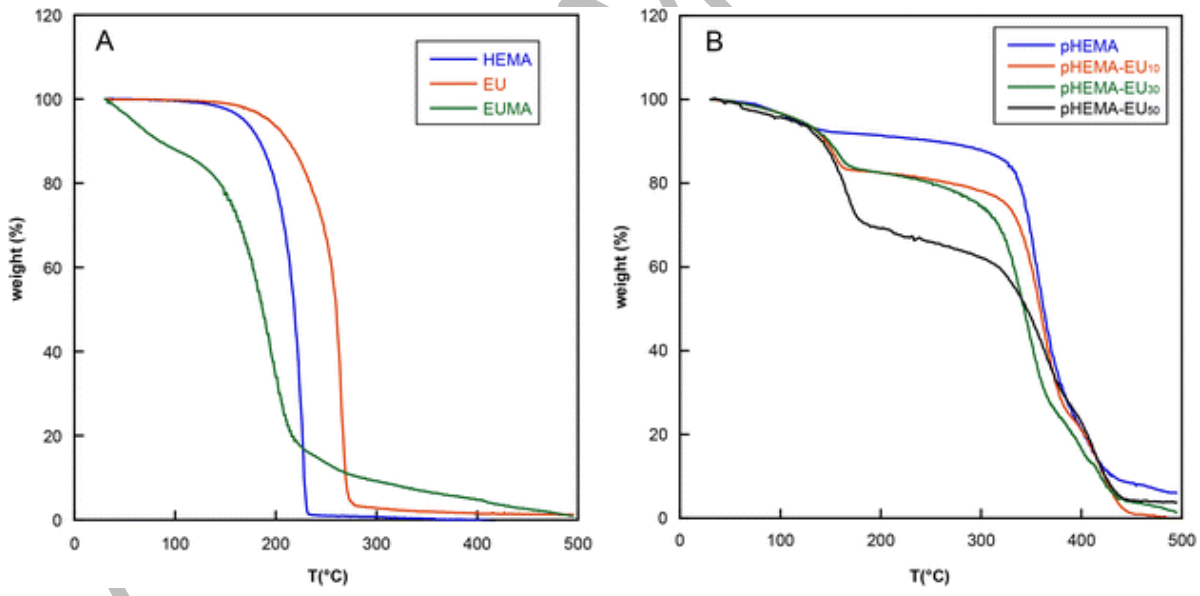


Fig. 5 Thermogravimetric curves of the starting compounds (A) and copolymers (B).

The pHEMA sample, after a first 5% weight loss at 100 °C due to the adsorbed water, remained stable up to *ca*. 250 °C (Fig. 5B). From the first derivative of the TGA curve, the degradation temperature was determined at 368 °C. The TGA curve of the pHEMA–EUMA copolymers showed an additional weight loss at *ca*. 160 °C, presumable related to the unreacted EUMA, suggesting an incomplete inclusion of EUMA in the polymer. The degradation temperatures of the copolymers were *ca*. 360 °C, slightly lower than neat pHEMA.

Differential scanning calorimetry showed that all of the samples are essentially amorphous polymers (Fig. 6). As for the neat pHEMA sample, the glass transition temperature (T_g) is not

easy to be determined but seems to be located at *ca.* 50 °C. In the literature, the glass transition temperature of pHEMA was found to vary from 50 to 90 °C according to the degree of crosslinking of the polymer chain, in turn depending on the polymerisation conditions.[30–32] The glass transition behaviour of pHEMA was significantly influenced by the EUMA content since the T_g value decreased from 50 to −85 °C when the EU unit varied from 0 (pHEMA) to 50 wt% (pHEMA-EU_{50}). The higher flexibility of the copolymers containing EU may be related to an increase in the excluded volume associated with the polymer chains, induced by the EUMA lateral arm.

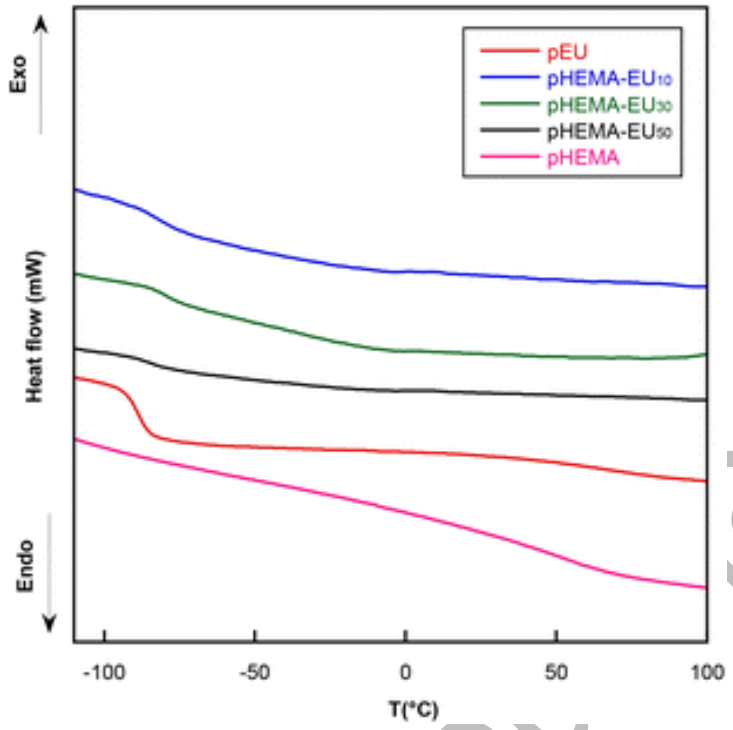


Fig. 6 DSC curves for pEU and pHEMA–EUMA copolymers in the second heating cycle.

A similar behaviour in the glass transition temperature in EU-based copolymers was reported by Al-Odayni *et al.* who copolymerised an EU-based methacrylate monomer with methyl methacrylate (MMA).[20] The authors related their findings to the reactivity ratio of the two monomers, which in turn affects the dominance of the different neighbouring interactions.[33]

3.4. Swelling of polymers in water

The swelling ability of the polymers in water decreased with the increase in the EU content (Fig. 7), presumably because of the hydrophobicity of the EU aromatic compound. As expected, pHEMA is an extremely hydrophilic polymer reaching a swelling degree at the equilibrium of *ca.* 300% (Table 1). Already the introduction of a low EU content (theoretically 10% in volume) reduced the maximum swelling degree by half. The pHEMA-EU_{50} with the highest EU content swelled *ca.* 70% (Table 1).

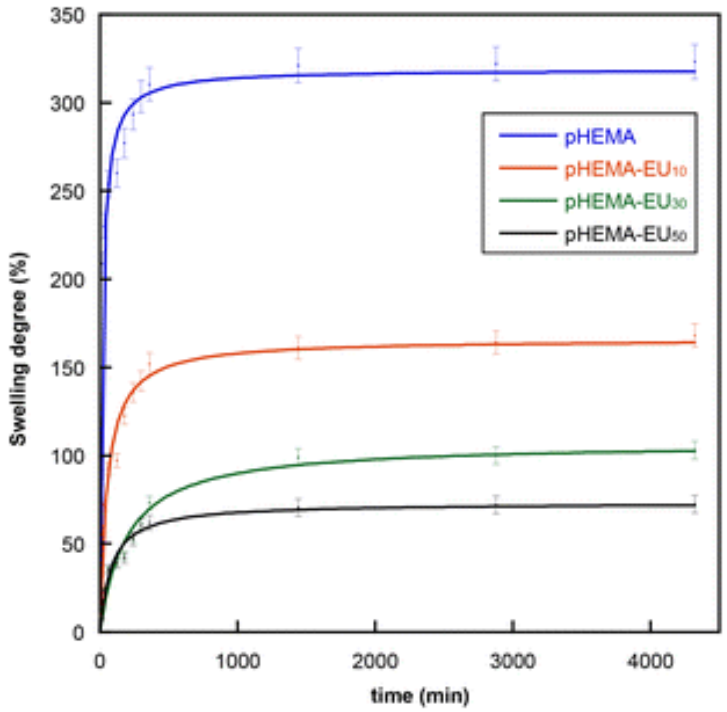


Fig. 7 Swelling of pHEMA and pHEMA–EUMA copolymers in water.

3.5. Rheological properties of polymers

Amplitude sweep tests were performed in the strain-control mode for strain amplitude in the range 0.1–2%, the oscillation frequency being 1 Hz. The resulting stress was recorded as a function of temperature and a deformation value of 0.5%, within the limits of linear viscoelasticity, was set for the following frequency sweep tests.

In Fig. 8A, the storage modulus (*G'*) and loss modulus (*G''*) of pHEMA and copolymers are reported *versus* the frequency.

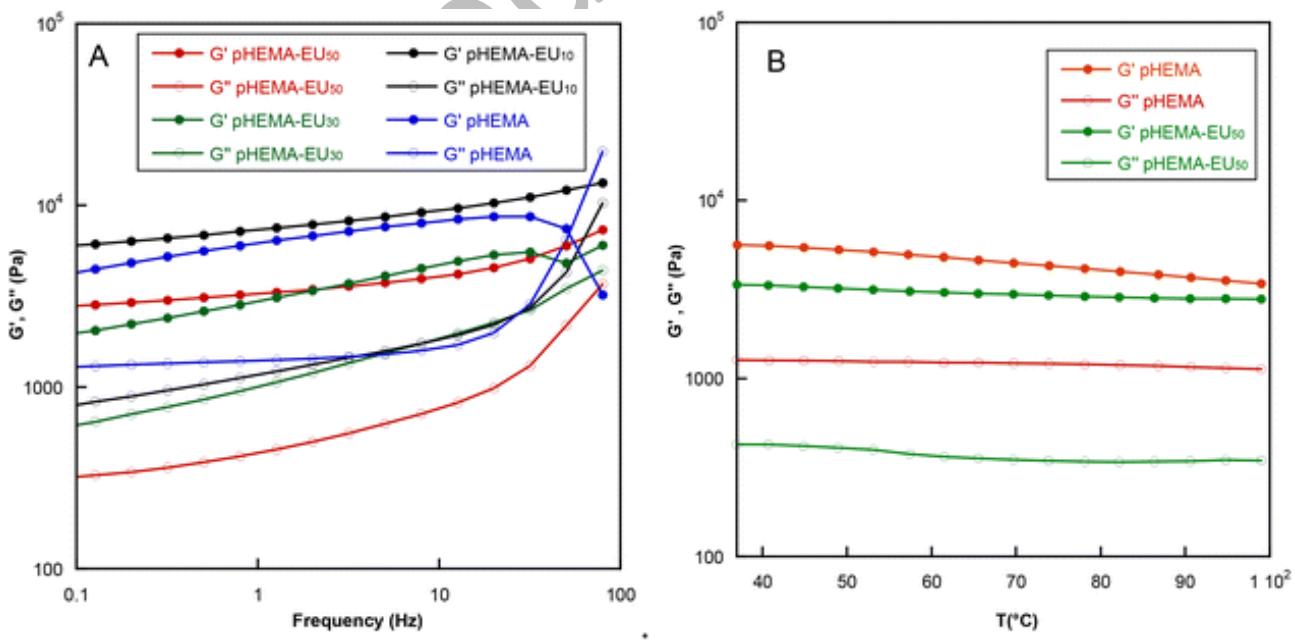


Fig. 8 Storage modulus (*G'*) and loss modulus (*G''*) of pHEMA and pHEMA–EUMA copolymers *versus* frequency at 37 °C (A). Storage modulus (*G'*) and loss modulus (*G''*) of pHEMA and pHEMA-EU$_{50}$ *versus* temperature at 1 Hz and a constant strain of 0.5% (B).

As far as pHEMA is concerned (blue curves), the value for the storage modulus dominates the loss modulus for a wide range of frequencies (up to *ca*. 50 Hz), which confirms the inherent good elastic properties of the obtained pHEMA sample. The storage modulus is approximately 5×10^3 Pa at a low frequency. For higher frequencies, ranging from 50 to 80 Hz, an inversion of moduli was observed suggesting material softening. This inversion of the two moduli was not observed for the pHEMA–EUMA copolymers, where both *G*' and *G*'' monotonically grew in all explored frequency ranges. The copolymer pHEMA-EU_{10} showed a *G*' value similar to the neat pHEMA. In contrast, the copolymer with the highest EU content, pHEMA-EU_{50}, had a storage modulus lower than pHEMA except at high frequencies (*ca*. 80 Hz), suggesting retainment of the elastic properties also at high deformation frequencies. This finding may be related to a significant presence of aromatic moieties which may contribute to the polymer mechanical stabilization by π–π stacking interactions.

The study of the rheological properties as a function of temperature did not show any structural transition of the material in the explored temperature range. As an example, in Fig. 8B, the trends of *G*' and *G*'' *versus* temperature for pHEMA and pHEMA-EU_{50} are reported.

3.6. Antioxidant activity of polymers

Eugenol is known for its many biological properties including the antioxidant and anti-inflammatory properties. In this work, the antioxidant properties of EU were assayed towards the radical DPPH and an EC_{50} value of 0.30 mmol per mmol DPPH was found.

The HEMA–EUMA copolymers are expected to exert activity. However, factors like steric hindrance and high molecular weight may negatively affect the ability of EU to interact with radicals when linked to a polymer backbone. In this study, antioxidant tests were performed on 5, 10 and 15 mg of solid samples, except for in the case of pEU for which an amount of only 5 mg was assayed due to a lack of material availability. In Fig. 9, the radical scavenging activity is reported for all of the samples. pHEMA did not show any significant antioxidant activity whereas the copolymers showed a marked ability to scavenge radicals even at low EU content (10%). This antioxidant property increased with the increase in EU content in the copolymer, again confirming a successful copolymerisation of EU and HEMA. The most active copolymer resulted to be the homopolymer pEU, for which *ca*. 85% percent of radicals was scavenged already with 5 mg of polymer.

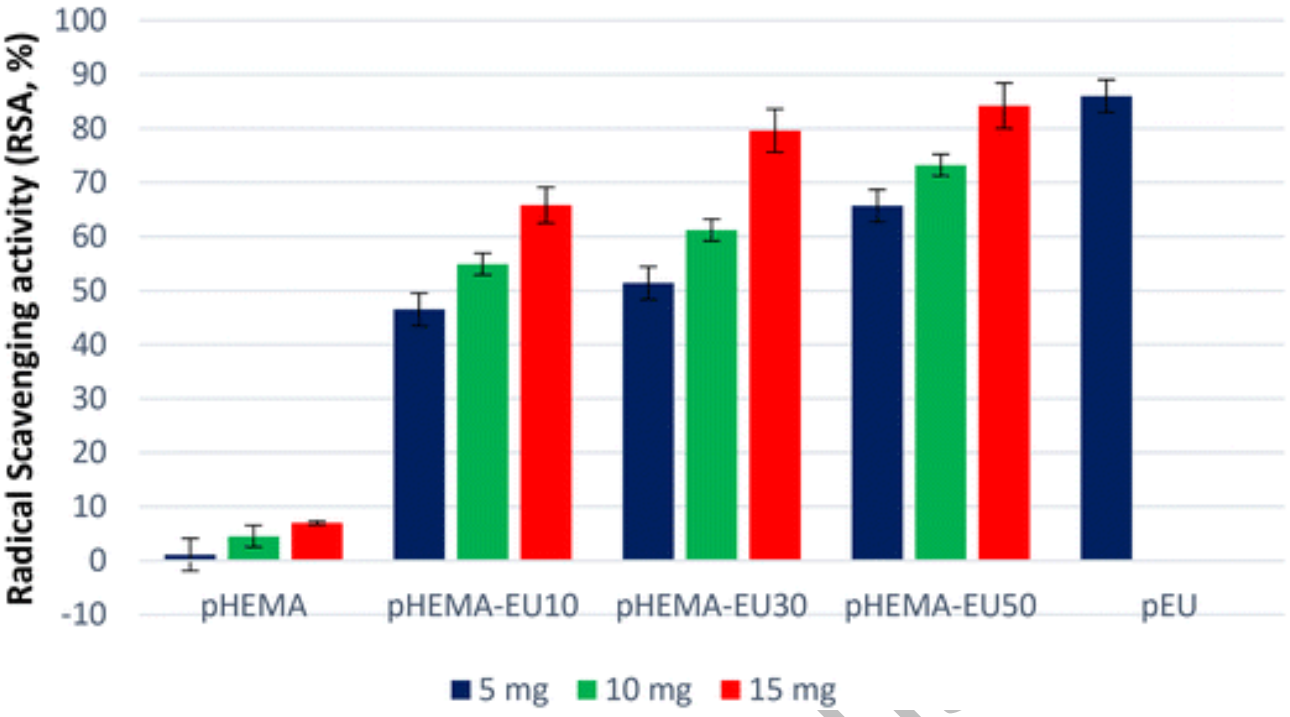


Fig. 9 Radical scavenging activity (RAS) of pHEMA, pEU and pHEMA–EUMA copolymers.

3.7. Antimicrobial activity of polymers

Many natural antioxidant phenolic compounds have been demonstrated to affect cell viability of different bacteria, including Gram positive and Gram negative,[34,35] suggesting the potential use of antioxidants for infection control.[19]

In Fig. 10, the bacterial growth percentage (BG, %) is reported. Also in this case, pHEMA did not show any activity while the copolymers decreased the BG to different extents in relation to the EU content in the polymer. However, for each polymer sample, the BG did not significantly depend on the amount of sample placed in contact with microorganisms, this finding is presumably related to the insolubility of the polymer.

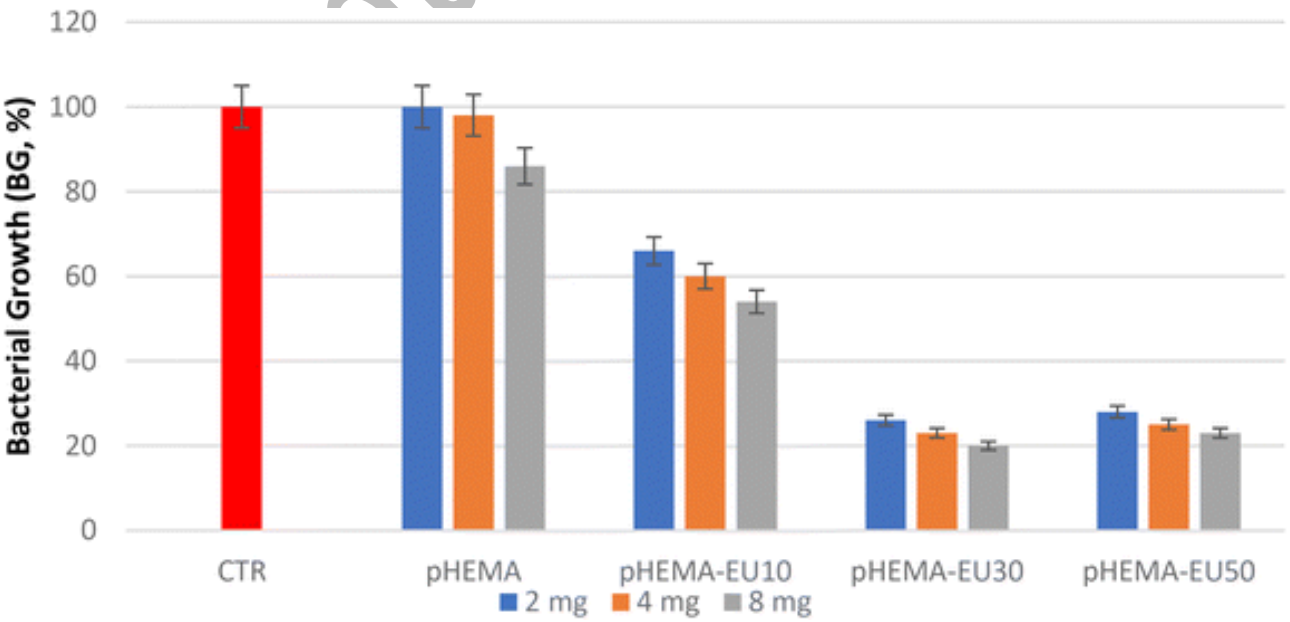


Fig. 10 Bacterial growth percentage of copolymers pHEMA–EUMA and pHEMA. *S. epidermidis* is the
Polymer amounts were suspended in 2 mL of MH broth.

Kwon and coworkers developed a model to elucidate the mechanism of antimicrobial action of some phenolic compounds (phenolic acids) by hypothesizing that they could behave as "proline analogues" and likely inhibit proline oxidation *via* proline dehydrogenase. Experiments suggested that the site of action of phenolic acids could the proline dehydrogenase, thus interfering with the bacterial metabolism.[36]

The good activity of eugenol in inhibiting the growth of Gram-positive bacteria found in this study has already been largely described in the literature.[37,38] In a recent study, among a number of screened phenolic compounds, eugenol showed the highest antibacterial activity *versus* *Pseudomonas aeruginosa* and *Staphylococcus aureus*, together with tannic acid, epigallocatechin gallate and rutin.[34] Different mechanisms have been hypothesised to explain the antimicrobial activity of eugenol. The most accredited is the ability to disrupt the cytoplasmatic membrane by interaction through its phenol group, thus increasing membrane nonspecific permeability and affecting the transport of ions and ATP.[38]

In this context, the development of eugenol-based antimicrobial polymers may contribute to potentiate the effects of eugenol on the bacterial cell surface. Indeed, the interaction of the macromolecular backbone with the bacterial cell may improve the outer membrane permeabilization, as described for many polymeric antibiotic adjuvants.[39]

4. Conclusions

In summary, we have demonstrated a novel synthetic strategy to obtain methacrylate polymers bearing phenol groups, starting from eugenol as a cheap and renewable feedstock. Overall, the synthesised eugenol-methacrylate monomer has the potential to be copolymerised with a wide number of different acrylate or vinyl monomers, thus allowing one to prepare a library of polymers with the desired physical and biological properties. In this work, it was successfully polymerised with HEMA, a biocompatible and hydrophilic monomer, largely employed in the biomedical field.

The obtained amorphous polymers were shown to be as thermally stable as pHEMA but more flexible (lower T_g) and hydrophobic (less swellable in water) than pHEMA. The rheological tests evidenced that for all of the copolymers the value of the storage modulus is higher than that of the loss modulus in all the explored frequency ranges (up to *ca*. 80 Hz). This suggests the inherent good elastic properties of the polymers, which were retained also at high deformation frequencies. This was not the same for pHEMA, for which an inversion of the storage and loss modulus was recorded at high frequencies.

Thanks to the presence of phenol groups in the side chain of the copolymers, the pHEMA–EUMA copolymers showed significant antioxidant and antimicrobial properties. To the best of our knowledge, there are no EU-based polymers showing such biological properties in the literature.

The cutting-edge antioxidant and antimicrobial properties shown by the prepared polymers open an interesting perspective towards the use of these polymers in different application fields.

Author contributions

Conceptualization, I. F. and L. M. M.; methodology, I. F. and L. M. M.; formal analysis, M.D.C.; investigation, M. D. C.; data curation, A. P.; writing – original draft preparation, I. F.; writing – review and editing, L. M. M. and A. P.; funding acquisition, I. F. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank the Sapienza University of Rome for financing the research (grant number: RP11916B459A8D63). The authors wish to acknowledge Professor Alessandra Gentili and Dr Lorenzo Antonelli of the Department of Chemistry of the Sapienza University of Rome for the LC-MS analysis.

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