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Perspective article

Synthesis and solution properties of poly(p,α dimethylstyrene-*co*-maleic anhydride): The use of a monomer potentially obtained from renewable sources as a substitute of styrene in amphiphilic copolymers

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ABSTRACT

The use of p,α-dimethylstyrene, potentially obtainable from renewable sources, as a substitute for styrene in the synthesis of amphiphilic copolymers is reported in this work. A series of novel poly(p,α-dimethylstyrene-*co*-maleic anhydride) (SMA) copolymers was synthesized, characterized, and studied as potential polymeric surfactants. After hydrolysis, the copolymers solution properties were compared to the similar and very well-known styrene-maleic acid copolymers. Both series of copolymers were synthesized using reversible addition-fragmentation chain transfer-mediated polymerization (RAFT), and a sample of poly(p,α-dimethylstyrene-*co*-maleic anhydride) was synthesized via classical free radical polymerization. The synthesized copolymers were studied from the point of view of their solution properties, with particular attention to the influence of the macromolecular and chemical structure on the surface tension of their aqueous solutions. Our results suggest that p,α-dimethylstyrene can be employed in copolymers with maleic anhydride, the resulting material being a valid alternative to SMA copolymers for various applications, such as emulsifiers and dispersants. Furthermore, the DMSMA series seems to be slightly more surface active than SMA.

1. Introduction

The exploitation of non-renewable resources and the constant increase of global pollution are stimulating scientific research towards finding new biobased alternatives to fossil-based chemicals and materials [1]. The development of new polymeric materials from renewable feedstocks has attracted great interest in recent years [2,3]. Two main strategies can be followed to perform the synthesis of biobased polymeric materials: 1) the use of bio-derived monomers, such as lactic acid, terpenes, fatty acids and others [4–7], or 2) the chemical modification of biopolymers and biomaterials [8–13]. In the context of the first approach, p,α-dimethyl styrene (DMS), can be evaluated as a potentially biobased promising substitute of styrene. This stems from the fact that DMS has the same carbon connectivity as some terpenes; therefore, it can potentially be synthesized from bio-based sources. For example, it has been reported that DMS can be formed by dehydrogenation of limonene or oxidation of p-cymene [14,15]. Limonene can be conveniently extracted from a bio-waste such as orange peel [16], and then

Abbreviations: RAFT, reversible addition-fragmentation chain transfer-mediated polymerization; DMS, p,α-dimethyl styrene; MAH, maleic anhydride; S, styrene; MS, α methyl styrene; SMA, styrene-*alt*-maleic anhydride; SMA-HYD, hydrolyzed styrene-*alt*-maleic anhydride; RDRP, reversible deactivation radical polymerization; CTA, control transfer agent; HPE, polyelectrolyte; CAC, critical aggregation concentration; DMSMA, poly(p,α- dimethystyrene-*alt*-maleic anhydride) copolymers; DMSMA-HYD, hydrolyzed poly(p,α- dimethystyrene-*alt*-maleic anhydride) copolymers; ATRP, atom transfer radical polymerization; CPY, 2-cyanobutan-2-yl 4-chloro-3,5-dimethyl-1H-pyrazole-1-carbodithioate; AIBN, 2,2′-Azobis(2-methylpropionitrile); SDS, Sodium dodecyl sulfate; THF, Tetrahydrofuran; Et_2O, Diethylethere; MeOH, Methanol; DMF, *N*,*N*-Dimethylformamide; NaOH, sodium Hydroxide; HCl, hydrochloridric acid; MCEBTTC, 2-butylthiocarbonothioylthio) propanoate trithiocarbonate; DMSO-d_6, Dimethyl sulfoxide-d_6 deuterated; D_2O, deuterium oxide; GPC, Gel permeation chromatography; wt%, weight percentage; M_n, molecular weight; TEM, transmission electron microscopy; DLS, dynamic light scattering; DST, Dynamic Surface Tension; CP, cloud point; $Đ$, dispersity; CMC, critical micelle concentration..

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can be either catalytically dehydrogenated to DMS with yields ranging from 20 to 60% [17,18], or oxidized to p-cymene and then to DMS with yields ranging from 8 to 27% [19]. However, it should be pointed out that this has not yet been implemented on large scale.

Despite its potential in terms of raw material usage, the polymerization of this monomer is challenging due to the presence of the methyl group in the alpha position, that strongly stabilizes the styrene derived radical. As it is already well-known for alpha methyl styrene, this class of monomers displays a really low ceiling temperature (~60 °C), which in turn makes cationic polymerization [20,21] the only feasible preparation route to obtain polymers with a relatively high molecular weight (typically in the order of 10^4 g/mol). A way to bypass this limitation is to employ them to synthesize alternating copolymers with specific monomers such as maleic anhydride (MAH) [22]. MAH also does not easily homopolymerize, but it is an electron-accepting monomer that cross-propagates well with an electron donor monomer such as, for example, styrene (S) or α methyl styrene (MS) [23].

The copolymerization of S and MAH leads to the synthesis of a well-known class of copolymers, styrene-*alt*-maleic anhydride (SMA), that has been broadly investigated over the years. They are mostly used industrially as emulsifiers and dispersants, but they are very versatile [24–26]. From these precursors, it is possible to obtain anionic, cationic or nonionic polymeric surfactants, respectively by saponification or by imidation of the anhydride functions [27]. Thanks to their surface activity, SMA surfactants have been employed as emulsion stabilizer in the synthesis of microencapsulated materials or in emulsion polymerization [28–32].

If a certain control over the polymer structure is desired, it is relatively easy to synthesize SMA copolymers by using reversible deactivation radical polymerization (RDRP) techniques, such as RAFT. [33,34] For example, different alternating copolymers with different S/MA ratio have been successfully synthesized in the presence of benzodithioate derivatives [35–37], S-dodecyl S′-2-(2,2-dimethylacetic acid) trithiocarbonate [38,39] or 2,2′-bis(propionic acid) trithiocarbonate as control transfer agent (CTA), with relatively low dispersity (1.1–1.3). [40]

As a consequence of the relatively low cost and functionalization strategies (vide supra) these polymers have been exploited in many fields such as chemical and biomedical engineering [41–44], energetic field [45–48], crude oil treatment [49,50] or as drug delivery systems. [51–54] Especially when biomedical applications are considered, it is important to notice that SMA polymers are already in clinical use, which reduces the time of clinical testing [55].

Seen the kaleidoscopic variety of applications of SMA copolymers, it is very relevant to find a suitable bio-based alternative to styrene for this class of polymer, with comparable properties, and this is the primary goal of this research.

From the point of view of their physico-chemical properties, polymers like SMA can be defined as hydrophobic polyelectrolytes (HPE) since they have several ionizable groups along the molecule that make them important for the variety of solution properties [56,57]. It is known that the amphiphilic character of a polyelectrolyte is due to formal charges along the chain, and hydrophobic groups on the polymer composition [58]. In light of this, by tuning the formal charge and/or the number and distribution of hydrophobic groups along the backbone, both the formation of intra- or inter-polymer aggregates are possible, with great impact on the final solution properties [56,59]. It has been reported that in the case of HPE with alternating structure, similar to the ones studied in this work, intra-polymer aggregates (pseudo-micelles) involving only individual polymer chains seem to be prevalent. [58,60,61] This means that it is not possible to talk about critical aggregation concentration (CAC) as it is normally done for associative amphiphilic copolymers. Since the aggregation behaviour comes from the balance between electrostatic repulsion and hydrophobic attraction, it is interesting to investigate if SMA and poly(p,α-dimethyl styrene-*alt*-maleic anhydride) copolymers (DMSMA) present any differences in this respect, as DMS is expected to be slightly more hydrophobic than S. Adsorption of surface-active agents at the interfaces is particularly significant when taking into account industrial applications, since surfactants are critical components for many products and processes [62].

DMS has a very similar reactivity to α-methyl styrene (MS), and in the literature, alternating copolymers of MAH and α-methyl styrene (MS) derivates have been synthesized using free-radical copolymerization with highly-ordered alternating structure. [23] Moreover, it was reported for all the MS derivates that the increase of the monomer to initiator ratio leads to a higher molecular weight, whereas the polymer dispersity remains consistent (~1.80 to 1.90).

In this work, a series of SMA and DMSMA copolymers with different molecular weight have been synthesized to evaluate their solution behaviour and establish if the substitution of S with DMS impacts the final properties of the polymer. The variation in molecular weight is also of interest, as it has been reported that SMA having different M_n display different surfactant- like properties that are crucial for biological applications [63]. We focused our attention on the solution properties of those polymers due to the lack of studies concerning the pristine hydrolyzed SMA and the hydrolyzed DMSMA. In this respect, dynamic light scattering and surface tension measurements were carried out. Preliminary cytotoxicity experiments were also carried out, which are relevant for several potential applications.

2. Experimental section

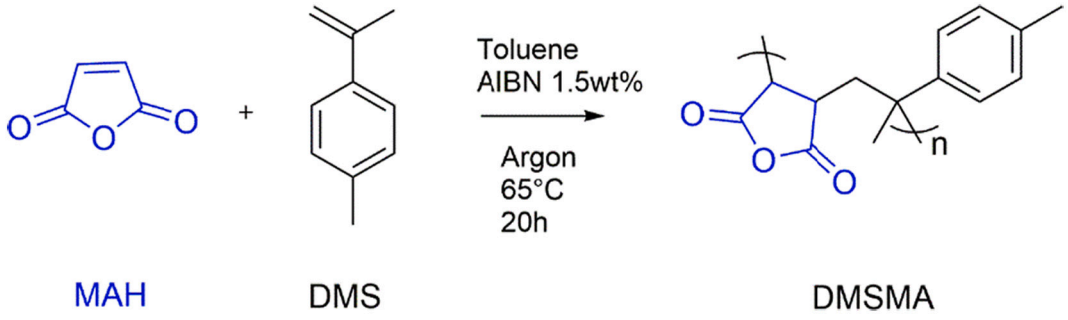
2.1. Materials

p,α-dimethyl styrene (DMS, Sigma-Aldrich, ≥95%, stabilized) was vacuum-distilled over CaH_2 and kept under nitrogen before use. Maleic anhydride (MAH, Sigma–Aldrich, puriss., ≥99.0%), 2-cyanobutan-2-yl 4-chloro-3,5-dimethyl-1H-pyrazole-1-carbodithioate (CPY, Boron Molecular, 95%), 2,2′-azobis(2-methylpropionitrile) (AIBN, Sigma-Aldrich, 98%), sodium dodecyl sulfate (SDS, ACS reagent, ≥99.0%), toluene (Sigma–Aldrich, anhydrous, 99.8%), tetrahydrofuran (THF, Sigma–Aldrich, ≥99.9%), diethylethere (Et_2O, Sigma-Aldrich), methanol (MeOH, Sigma-Aldrich 99.8%), 1,4-dioxane (Sigma-Aldrich, anhydrous, 99.8%), *N*,*N*-dimethylformamide (DMF, Sigma-Aldrich, for HPLC, ≥99.9%), sodium hydroxide (NaOH, ACS reagent, ≥97.0%, pellets) and hydrochloridric acid (HCl, ACS reagent, 37.0%) were used as received. 2-butylthiocarbonothioylthio) propanoate trithiocarbonate (MCEBTTC) was synthetized in this work according to the procedure reported in the supporting information of this paper. Dimethyl sulfoxide-d_6 (DMSO-d_6, anhydrous, 99.9 atom % *D*, Sigma-Aldrich) was used as a deuterated solvent for proton nuclear magnetic resonance (^{1}H NMR) studies.

2.2. Methods

2.2.1. Synthesis of p,α-dimethylstyrene-*co*-maleic anhydride polymer (DMSMA) via free radical polymerization

The p,α-dimethyl styrene-*co*-maleic anhydride copolymer (DMSMA) made via free radical polymerization was synthesized according to the following procedure (Scheme 1). DMS (6 g, 45.4 mmol), MAH (4.45 g, 45,4 mmol) and deoxygenated toluene (50 mL) were introduced under argon in a 100 mL round-bottomed flask equipped with a magnetic stirring bar and a reflux condenser and purged with argon for 40 min at room temperature. After the solution was deoxygenated with argon, the AIBN (0.157 g, 1,5 wt% with respect to the monomers mass) was added, and the reactor was put in an oil bath set to a temperature of 65 °C. After 20 h, the reaction was stopped and cooled down, introducing air and diluting with around 20 mL of THF. The THF solution was precipitated in a twentyfold excess of diethyl ether. The precipitate was washed with Et_2O and dried overnight at 60 °C, affording a whitish solid. The polymers were characterized by ^{1}H NMR, gel permeation chromatography (GPC) and Fourier-transform infrared spectroscopy (FTIR).



Scheme 1. Synthesis of p,α-dimethylstyrene-*co*-maleic anhydride polymers (DMSMA) via free radical polymerization.

*2.2.2. Synthesis of Poly(styrene-**co**-maleic anhydride) (SMA) via RAFT*

Typically, 3.00 g of S (28,8 mmol), 2.83 g of MAH (28,8 mmol) and an amount of CPY based on the aimed molecular weight were added in a 100 mL three-necked round bottom flask. Then, 28,8 mL of 1,4 dioxane and the amount of AIBN (0.5 eq. to the RAFT) were added to the flask. The solution was purged with argon for 45 min under stirring. After that, the round bottom flask was sealed and heated at 65 °C. Aliquots of the reaction solution were taken at regular time intervals to follow the reaction kinetics, and analyzed by ^{1}H NMR. The monomer conversion was determined via ^{1}H NMR spectroscopy by dissolving the crude solution in DMSO-d_6.

The resulted polymers were precipitated in Et_2O, dried under vacuum and characterized by ^{1}H NMR, GPC and FTIR.

*2.2.3. Synthesis of Poly(p,α-dimethyl styrene-**co**-maleic anhydride) (DMSMA) via RAFT*

3.80 g of DMS (28,8 mmol), 2.83 g of MAH (28,8 mmol) and 0.17 g of MCEBTTC (0,66 mmol) were added in a three necks round bottom flask of 100 mL. Then, 28,8 mL of 1,4 dioxane and a weighted amount of AIBN (0.5 eq. to the RAFT) were added to the flask. The solution was purged with argon for 45 min under stirring, then the system was heated at 65 °C. Aliquots of the reaction solution were taken at regular time intervals to follow the reaction kinetics, and analyzed by ^{1}H NMR. The monomer conversion was determined via ^{1}H NMR spectroscopy, by dissolving the crude solution in DMSO-d_6. The resulted polymers were precipitated in Et_2O, dried under vacuum and characterized by ^{1}H NMR, GPC and FTIR.

*2.2.4. Hydrolysis of Poly(styrene-**co**-maleic anhydride) (SMA) and Poly (p,α-dimethylstyrene-**co**-maleic anhydride) (DMSMA)*

All the polymers synthesized in this work were hydrolyzed according to the following procedure. Typically, 4 g of polymer were added to 80 mL of 2 N NaOH solution and stirred overnight at room temperature (Scheme 4). Then the excess base was removed by dialyzing against MilliQ water, changing the water at least 3 times over a period of 2 days. The dialyzed solution was then dried at 60 °C under vacuum for 24 h. The products were recovered as glassy transparent whitish solids and characterized by GPC and FTIR. It is important to mention that the basic solutions of DMSMA series after hydrolysis, presented a precipitate (~30 wt%) that was insoluble in all the solvent tried (DMF, DMSO, $CHCl_3$). Due to their insolubility, the solids were analyzed only by FTIR (Fig. S1), but their structure remains undetermined. The only hypothesis we can make, based on the absence of carbonyl peaks and the possible presence of aromatic ones, is that these are cross-linked derivatives of p,α-dimethylstyrene, but this was not further investigated because we judged it beyond the scope of this work. In any case, only the soluble part after hydrolysis was used for the solution studies.

2.3. Characterization

The p,α-dimethyl styrene-*co*-maleic anhydride polymers were characterized by ^{1}H NMR, GPC and FTIR analysis. The NMR spectra were recorded on a Varian Mercury Plus 400 MHz spectrometer using DMSO-d_6 as solvent. The FTIR spectra were recorded with a Shimadzu IR-Tracer-100 with golden gate diamond attenuated total reflectance (ATR) sample unit in the range 4000 cm^{-1} to 500 cm^{-1}, at a resolution of 4 cm^{-1} averaged over 64 scans. The molecular weights (M_n number and M_w weight-average molecular weights) and the dispersity ($Đ$) of the samples were determined by GPC using DMF (containing 0.01 M LiBr) as the solvent in a Viscotek GPCmax instrument equipped with model 302 TDA detectors and two columns (Agilent Technologies-PolarGel-L and M, 8 μm, 30 cm) at a flow rate of 1.0 mL/min and 50 °C. Low dispersity polymethyl methacrylate (PMMA) standards (Polymer Laboratories) were used for constructing a universal calibration curve. For sample preparation, the purified dry samples were dissolved in DMF (containing 0.01 M LiBr). Once the samples were completely dissolved, they were filtered through a polytetrafluoroethylene (PTFE) syringe filter (Minisart SRP 15, Sartorius stedim biotech, PTFE membrane filter; pore size, 0.2 μm; filter diameter, 15 mm) and analyzed by GPC using a 100 μL injection volume. The collected spectra were analyzed with the use of an OmniSEC instrument (v5.0) (Malvern). The molecular weight of hydrolyzed polymer was measured using an Agilent 1200 Series, equipped with three PSS Suprema columns: 100; 1000; 3000 Å 300 × 8 mm 10 μm. Detection was made with a refractive index detector (RID). The samples were eluted with 0.05 M aqueous $NaNO_3$ at a flow rate of 1 mL/min. Molecular weights and $Đ$ were determined using the software PSS WinGPC Unity from Polymer Standard Service. pH measurements were carried out using an S2 seven2Go pH Meter, adding HCl (0.5 M) to the polymer solution (1 wt%) until the cloud point was found. The surface tension of water polymer solutions at different concentration and at pH of 10.5 (value obtained after dissolution of the fully neutralized polymer) was measured with an OCA 15EC tensiometer from Dataphysics, using the pendant drop method. Dynamic light scattering measurement (DLS) of the solution at different concentration in water were carried out using a Malvern Zetasizer Ultra instrument.

Polymer solutions at pH of 10.5 and in the concentration range of 5–30 wt% were analyzed by cryogenic-transmission electron microscopy (cryo-TEM) in order to evaluated the presence of supramolecular aggregates. A drop of random copolymer solution was placed on a glow discharged plain carbon coated 400 mesh copper grid. The samples were examined in a FEI T20 electron microscope operating at 200 keV. Images were recorded on a slow scan charge-coupled device (CCD) camera. Fluorescence spectra of water polymer solutions, having different polymer concentrations and pH of 10.5, were recorded with a Jasco FP-8300 fluorimeter (right angle geometry, 1 cm × 1 cm quartz cell) using the following conditions: excitation at 333 nm, slit width 5 nm for the excitation, and 1 nm for the emission. The intensities of the bands I_1 at 372 nm and I_3 at 383 nm were then evaluated, and their ratio was plotted vs. the polymer concentration. Each sample was prepared in order that the final concentration of pyrene (previously dissolved in MeOH) in the water polymer solution was 2.5 × 10^{-7} M.

Finally, cytotoxicity tests were carried out as follow. The polymer

solutions were tested with mouse L929 fibroblasts. Solutions were added to the growth medium, ensuring that at least 90% of the liquid consisted of the medium. Cells were cultured for 24 h in 12-well plates. Then the polymer-containing medium was added and cells were exposed to the polymer for another 24 h and 72 h. For this 2-fold further serial dilutions were used. In order to create sterile polymer solutions, the received solutions were centrifuged at 13.000 *g*, as filtration was not possible.

3. Results and discussion

3.1. Polymers synthesis

A series of SMA and DMSMA copolymers with different average molecular weights were synthesized. As mentioned in the introduction, RAFT polymerization was used to ensure the formation of linear structures with low dispersity, and to obtain polymers with similar molecular weight. This allows to better evaluate the potential differences linked only to the use of DMS instead of S, and no other structural parameters as the molecular weight or architecture [64].

As reported in Table 1, SMA and DMSMA were synthesized using similar conditions. A DMSMA copolymer was synthesized via free radical as a control. All the polymers were characterized by ^{1}H NMR, FTIR, and GPC (supporting information, Fig. S2, Figs. S3 and S4). Already in the synthesis of the two series of polymers, it is possible to highlight some differences between the use of S and DMS. First of all, the two series were synthesized using different RAFT agents (Schemes 2 and 3). The RAFT agent used for SMA, CPY, resulted ineffective for DMSMA, since for the latter M_n did not increase with conversion, where MCEBTTC provided better results. Kinetic plots (Fig. 1) indicate that the concentration of radicals remains constant at short times for both polymerizations (pseudo-first order), but for DMSMA the linearity is lost at longer times, indicating possibly more termination events for this system. This may be due to the use of different RAFT agents, but this and other mechanistic aspects were not further investigated.

The conversion was monitored by ^{1}H NMR, based on the disappearing of the proton signals associated with the vinyl bond at ~5.2 ppm (Fig. S2) of the styrenic monomer. The series with S reaches higher conversion compared to DMS.

The GPC traces of SMA polymers appear to be relatively narrow and monomodal, in line with what has reported in the literature [33,37,65], except for the polymer with higher targeted molecular weight SMA 50KDa (Fig. S4), which has a $Đ$ of 1.89. The analogous GPC traces of the DMSMA series are broader and bimodal (Fig. S4). As already indicated above, MCEBTTC proved to be a more suitable RAFT agent than CPY for this specific polymer, but still not optimal in achieving narrow MWD and values of Mn close to the theoretical ones.

The bimodal nature of the DMSMA GPC traces is more pronounced when high molar masses are synthesized. A possible explanation can be found in the fact that targeting higher molar mass, the monomer/RAFT agent ratio increases as well, resulting in poorer control.

Although the dispersity of DMSMA series is broader than the SMA series, it is still considerably lower than the DMSMA copolymer synthesized by free radical polymerization, used as control (Table 1). These data do not indicate overall a good control and livingness of the polymerization process. Nevertheless, and as opposed to free radical polymerization, the synthetic method used provided polymers with comparable molecular weights and relatively narrow dispersity, enabling comparisons about solution properties, discussed in the next section. Moreover, with our synthetic strategy, we can be reasonably certain that the polymers prepared in this work possess, at least predominantly, a perfectly alternate structure. In the case of DMSMA polymers, this comes from the fact that neither monomers are able to self- propagate via radical mechanisms [23], while for SMA polymers, where styrene could in principle self-propagate, perfectly alternate structures have been shown to be preferred at low temperature and when styrene is not used in excess [37], both conditions being verified here.

After synthesis, the copolymers were hydrolyzed using aqueous NaOH, purified by dialysis, and analyzed by FTIR and GPC in aqueous media (Figs. S5 and S6). The GPC traces show that the two series of polymers are comparable in terms of relative M_n (Table S1) between polymers with the same theoretical M_n showing the same trend. Interestingly, the DMSMA series of polymers contained an insoluble fraction after hydrolysis, while the SMA series is completely soluble. Based on the FTIR spectra (SI, Fig. S1) we suspect that this residual solid consists of cross-linked material, containing mostly the DMS monomer. This is plausible since DMS, as opposed to S, does contain sites that can transfer and propagate radicals (e.g., the *p*-methyl group). However, we did not further investigated this insoluble solid.

3.2. Polymers solution studies

It is often reported in literature that SMA-HYD copolymers can give self-assembly in water; however, those polymers are usually not perfectly alternate but contain a block of S, responsible for the aggregation [33,66]. For alternating copolymers, as it is supposed to be in our case (see discussion in previous section), aggregation is not expected to happen.

As a matter of fact, for alternating SMA-HYD, no relevant aggregation studies are reported to the best of our knowledge. Some authors

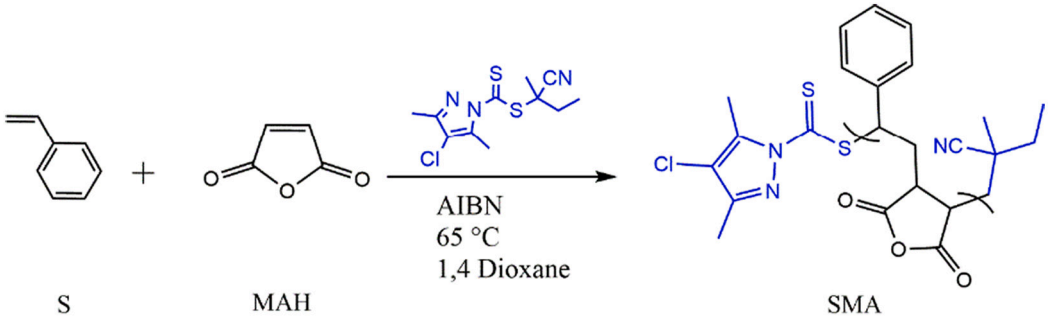
Table 1
Conditions and results of the polymerizations carried out in this work.

Sample name	CTA	Conv[a] (%)	$[M]_0$:$[CTA]_0$:$[I]_0$[b]	M_n^{theo} [c] (Kg•mol^{-1})	M_n^{expe} (Kg•mol^{-1})[d]	$Đ$	k_p [e] ($\times 10^{-3}$ h^{-1})
SMA 10 kDa	CPY	93	1.10: 0.020: 0.001	10.6	15.7	1.33	117.6
SMA 25 kDa	CPY	99	1.10: 0.008: 0.001	27.8	35.4	1.29	205.1
SMA 50 kDa	CPY	96	1.10: 0.004: 0.001	53.6	37.6	1.81	142.3
DMSMA 10 kDa	MCEBTTC	79	1.10: 0.023: 0.012	9.0	28.4	1.98	89.2
DMSMA 25 kDa	MCEBTTC	70	1.10: 0.009: 0.004	20.0	56.4	1.89	56.0
DMSMA 50 kDa	MCEBTTC	55	1.10: 0.005: 0.002	28.1	72.6	1.88	45.0
DMSMA 20 kDa FR	–	74	0.91: -: 0.02	–	35.3	3.84	–

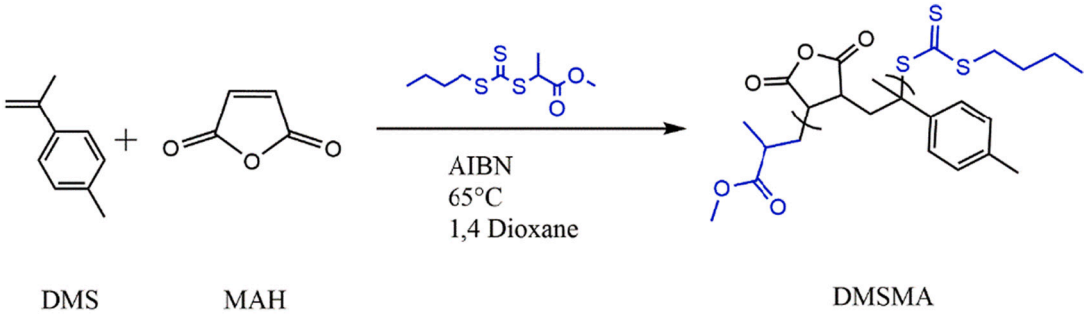
[a] Conversions were determined by ^{1}H NMR spectroscopy.
[b] $[M]_0$ refers to a 1:1 mixture of the two monomers
[c] M_n (theory) at experimental conversion values having an exactly alternating structure based on the following equation:

$$M_n^{th} = \left(Conv \times \frac{W_m}{n}\right) + M_{CTA}$$

where W_m is the initial monomer weight, n is the moles of the CTA, M_{CTA} is the molecule weight of CTA and Conv is monomer conversion.
[d] Based on PMMA standards.
[e] Overall polymerization constant rate (k_p) based on the slope of the kinetic studies (Fig. 1).



Scheme 2. Synthesis of styrene-co-maleic anhydride copolymers (SMA) via RAFT.



Scheme 3. Synthesis of p,α-dimethylstyrene-co-maleic anhydride copolymers (DMSMA) via RAFT.

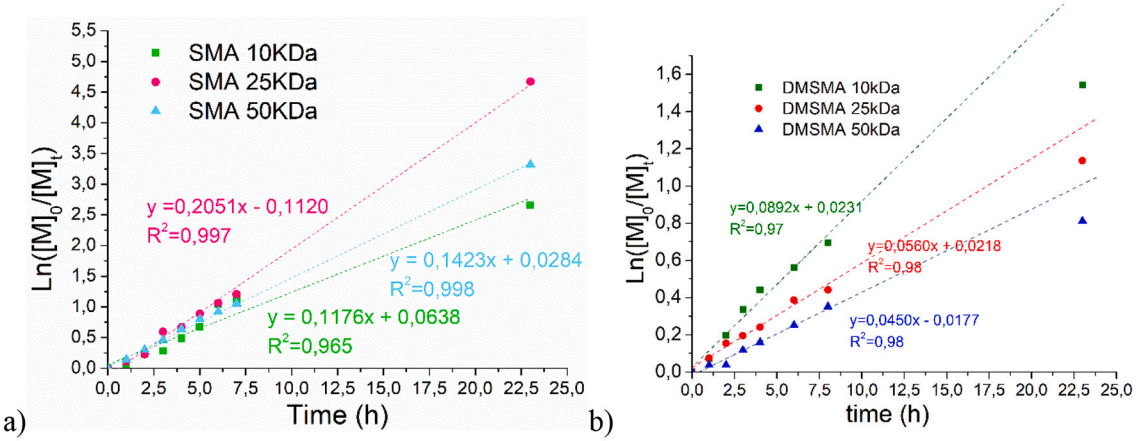
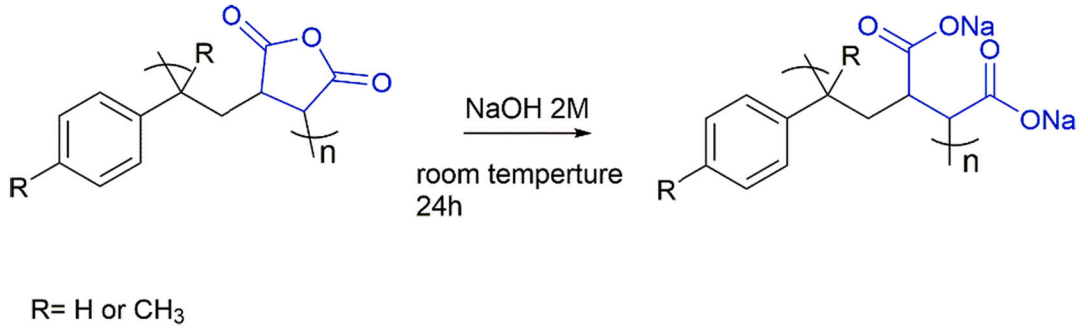


Fig. 1. Kinetics plots (1st order) of SMA (left) and DMSMA (right) at different targeted M_n via thermo initiated RAFT.



Scheme 4. Hydrolysis of poly(styrene-*co*-maleic anhydride) (SMA-HYD) and Poly(p,α-dimethyl styrene-*co*-maleic anhydride) (DMSMA-HYD).

reported SMA-HYD aggregates constituted by SMA/lipids to form lipid disks [24,38].

In order to investigate the nature of the possible aggregation behaviour of alternating SMA-HYD and DMSMA-HYD, DLS measurements were carried out (Figs. S7 and S8). Although aggregation is not expected, as explained above, DLS seems to detect the presence of structures in the 1–4 nm range at low concentrations. As a first impression, it seems that there are differences in the aggregate dimension of the two series. However, these differences are relatively small and they are observed for the solutions at higher polymer concentration (above 20 wt%), which may derive from artefacts in the measurements due to the highly overlapping concentration regime, where polymer chains interact with each other [67]. Due to the possibly unreliable DLS data, to further understand the possible aggregation behaviour in the high concentration regime, measurements of fluorescence were carried out on the polymer solutions at different concentrations using pyrene as a fluorescent probe. In the years, the use of pyrene as a fluorescent probe has been a helpful tool to prove the presence of polymer aggregates with hydrophobic domains in water [68–70]. Depending on the pyrene microenvironment, the ratio between two of its characteristic emission peaks at 372 nm (I_1) and at 383 nm (I_3) changes from a higher value (pyrene in the hydrophilic domain) to a lower one (pyrene polymer aggregate microdomain) (Fig. S9). This ratio plotted as logarithmic function of the polymer concentration (Fig. 2), suggests a gradual change of the surrounding environment of the pyrene with polymer concentration. However, a plateau in the I_1/I_3 ratio, expected in the case of micellization, is not reached. It must be noted that this value can be altered because at higher polymer concentration, the I_3 signal overlaps with the polymer emission signal (380 nm −500 nm), making its measurement of difficult interpretation (Fig. S9). In any case, there is no sufficient evidence of aggregation, in line with what reported in the literature for similar polymers [56].

This was further confirmed by cryo-TEM (Fig. S10). Also in this case, no visible aggregates were observed, which confirms that DLS results at high concentration were artefacts.

All the techniques used bring us to the conclusion that the alternating SMA-HYD and DMSMA-HYD do not self-assemble in interpolymer aggregates in solution, even in a very concentrated regime. Therefore they appear to behave as polyelectrolytes with low hydrophobicity [70].

As the main possible applications of such polymers are related to surfactant properties, the surface tension was investigated, in relation to the presence of DMS instead of S, and the different molecular weight [62].

Fig. 3a display that for both series, the polymers with lower M_n show a slightly more pronounced decrease in the surface tension with concentration (Fig. 3a). Moreover, although the surface tension values measured for the SMA-HYD and DMSMA-HYD copolymers are similar, it seems that the use of DMS may give slightly lower surface tension values. These differences are minor but significant, as confirmed by a T-student test run on all the samples (SI, Table S2). Indeed, the only two samples comparisons that failed the test were SMA-HYD 25KDa-SMA-HYD 50KDa and the DMSMA-HYD 10KDa-DMSMA-HYD 50KDa, that means that difference between SMA-HYD and DMSMA-HYD are statistically real.

These differences, albeit not very significant in absolute value, can be explained by two factors: the first one is the higher hydrophobicity of DMS compared to S, as it is reported that this should correspond to increased surface activities in general [70,71].

The second factor could be linked to the fact that DMSMA-HYD series have a broader molar mass distribution ($Đ$) than the SMA-HYD one. An higher number of shorter polymer chains may better arrange at the interface, resulting in higher surface activity. This hypothesis is in line with the measurement of surface tension of DMSMA-HYD obtained by free radical polymerization. This polymer has significantly higher dispersity than the series synthesized by RAFT, meaning that it contains a high number of short chains that may be responsible for better surface activity. Analogous behaviour has been observed in a previous study on amphiphilic random copolymers based on styrene and (meth)acrylic acid published by our group [70].

The prepared polymeric surfactants are less surface active than molecular surfactants, as expected (see the comparison with SDS, Fig. 3b), and the measured values are in line with the one reported in the literature for similar SMA-HYD systems [33].

Due to their slower dynamics, polymeric surfactants are expected to reach equilibrium surface tension at longer times than molecular ones, ranging from milliseconds to several hours, depending on different factors as chain length, size, and charge of the polar head, nature of the liquid, and also the presence of other additives [72,73]. For this reason, Dynamic Surface Tension (DST) of the prepared polymers was measured over a time interval of 9 min (Fig. 4 and S12). The DST trend results to be independent of the polymer concentration, however comparing the trend of different polymers at 5 wt% (Fig. 4), it is possible to add further considerations. While SDS (used as a reference for low molecular weight surfactant) reaches the equilibrium absorption almost immediately, the polymers all show slower dynamics, with values for lower M_n decreasing faster than for higher M_n. No significant differences are observed between the polymers containing S or DMS.

SMA-like polymers have been characterized in literature for their pH-dependent properties and aggregation behaviour [59]. This expands the realm of possible applications, in particular in the biomedical field [38]. Therefore, we considered of interest to preliminarily observe the pH-dependent behaviour for the polymer prepared via free radical polymerization, selected as the most surface active.

A polymer solution of DMSMA-HYD FR at 1 wt% was titrated using

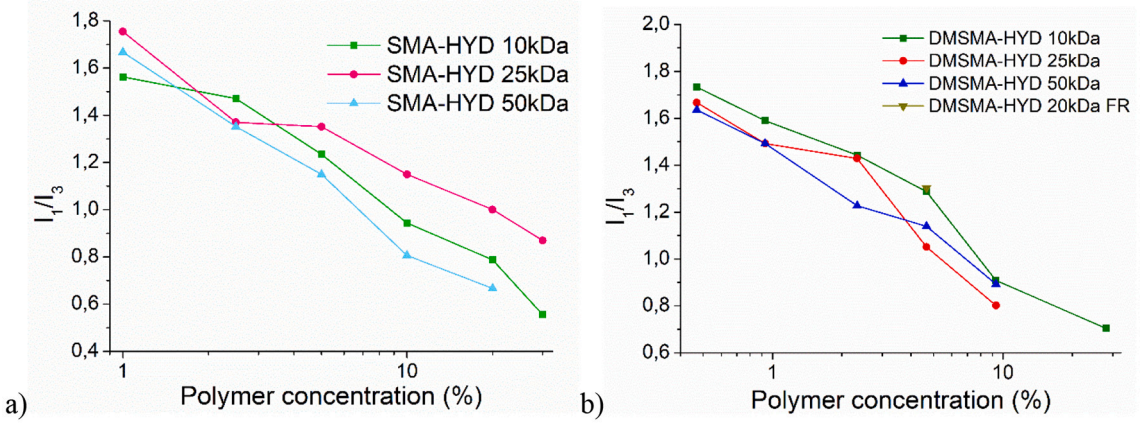


Fig. 2. Weight polymer concentration as a function of I_1/I_3 ratio for a) SMA-HYD series and b) DMSMA-HYD series.

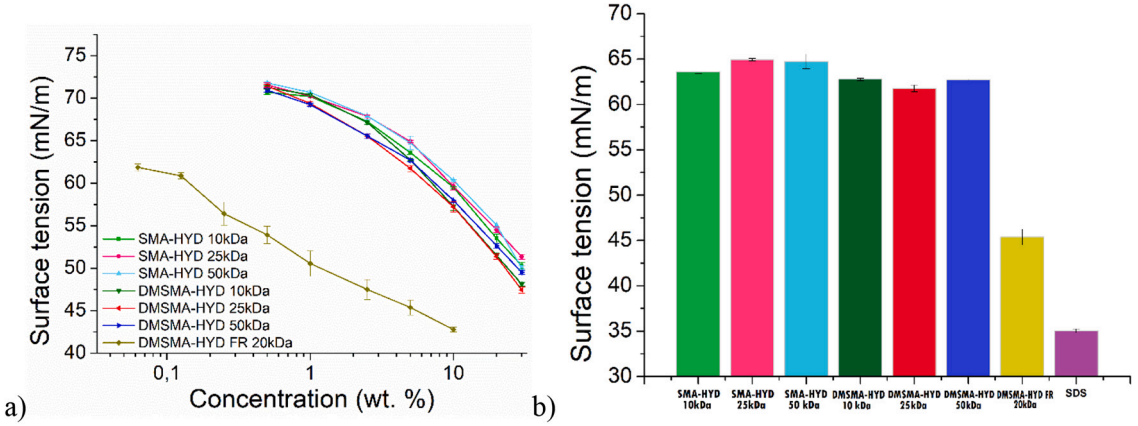


Fig. 3. Comparison of the Surface tension at different polymers concentration of SMA-HYD and DMSMA-HYD series (a); comparison of the surface tension values at 5 wt% of the different polymers synthetized in this work and the molecular surfactant SDS (b). Magnification of the Fig. 3b to better appreciate the error bars on the measurement is reported in SI (Fig. S11).

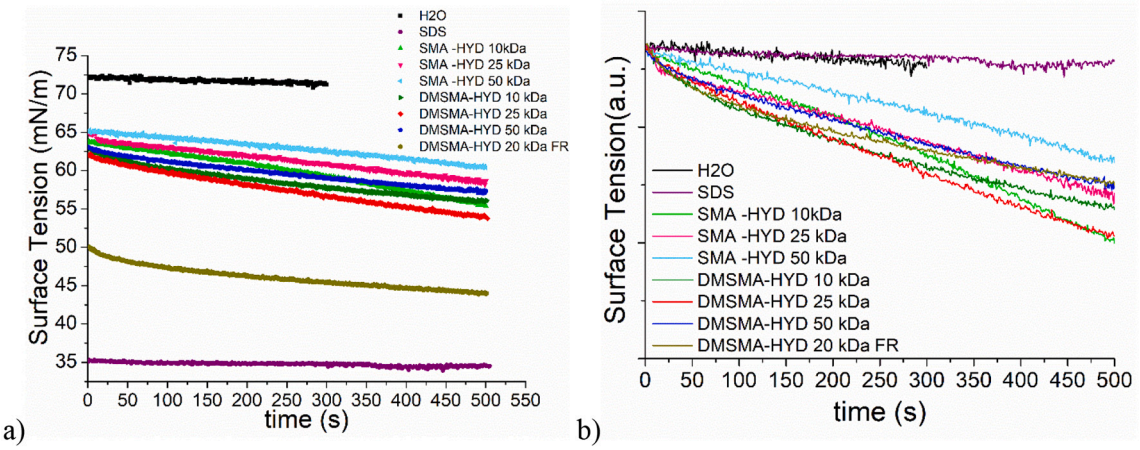


Fig. 4. Comparison of the Dynamic Surface tension overtime at 5 w.% between the polymer surfactants and the SDS (a); Comparison of the trend of the dynamic surface tensions of the graph 4a (b).

0.5 M HCl (Fig. S13), finding a cloud point at a pH of about 2, in line with what reported in the literature for SMA-HYD copolymers [74].

For the same polymer, we measured surface tension at two different pH: completely neutralized form (pH = 10.5), and at 65% of the protonated group, (obtained by addition of a specific amount of HCl, (pH = 9.5). Typically, SMA-HYD is employed in the pH range 7,5-8,0, where it is mainly deprotonated and completely soluble [74]. Additionally, the comparison between the same polymer in its salt form and in its partially protonated form (Fig. S14), shows as expected a decrease of the surface tension value, and the DMSMA-HYD (pH = 9.5) seems to have an apparent CMC above 0.25 wt%.

As many target applications of this kind of polymers are in biomedical and biological fields, we considered also interesting to have some preliminary information on cytotoxicity of the prepared polymers, particularly if the substitution of S with DMS may have some influence on behaviour with eukaryotic cells. The results of the incubations for DMSMA-HYD highlights that fibroblasts responded very well, with many cells being vital with good morphology.

The usual way of preparation includes an extraction test, in which the solids are brought in the aqueous medium for 24 h. The solids completely dissolved in the aqueous medium over 24 h at 37 °C. Therefore, it was decided to prepare a serial two-fold dilution series with these solutions and expose fibroblasts to these solutions. Cells were incubated for 24 h under normal conditions before exposure to solutions varying from 1:2 to 1:32 dilutions. After 24 h and 72 h of exposure, a cytotoxicity test based on the cleavage of the yellow tetrazolium (XTT) was applied to the cells. Cells died with the 1:2 dilution at day 1 when exposed to DMSMA-HYD, and also with the 1:2 and 1:4 dilutions at day 3. Moreover, as expected, the cytotoxicity increased with the polymer concentration. One particular reason for this can be the higher pH. As the starting pH of our polymer solutions is at a value of 10.5, the pH should be corrected towards pH 7.4, in this case, by using 3 N HCl. This, however, immediately led to flocculation of the dissolved polymer, rendering the sample useless. Most likely, the reason why polymer flocculation is observed, could be linked to the polymer's solubility in medium cells. The presence of different salts, fundamental for cells survival, can affect the HPE solubility at that pH. So, incubations had to be done at a pH that may have been too high for the cells to endure (pH = 10.5). For the extraction test, the data are shown in Fig. 5.

Exposure to the mentioned concentrations results in a clear reduction of metabolic activity compared with the medium control (0 mg/mL of polymer). Also, the DMSMA-HYD seems to affect the cell morphology significantly. It is interesting to see that the 72 h incubation data indicate a more dramatic impact on the cells than the 24 h incubation data, which is marked most by a lack of activity increase from 1 to 3 days of incubation with DMSMA-HYD. These observations are supported by fluorescent images of the cells stained with 4′,6-diamidino-2-phenylindole (DAPI) (nuclei) and fluorescein isothiocyanate (FITC)-phalloidin (actin cytoskeleton) (Fig. S15 for 24 h exposure and Fig. S16 for 72 h exposure). The toxicity is evident for the highest concentration of

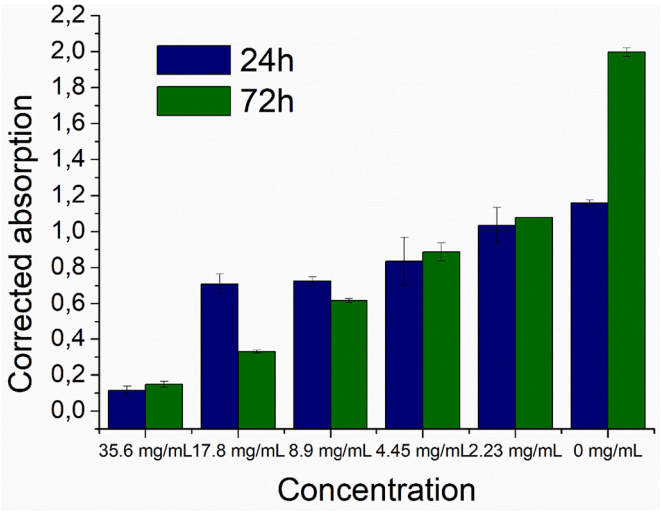


Fig. 5. XTT conversion (metabolic activity) by L929 fibroblasts after 24 h (blue) and 72 h (green) exposure to extracts of DMSMA-HYD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DMSMA-HYD after 24 h; however, decreasing concentrations affect cell morphology much less. Nevertheless, after 72 h of exposure also the other, lower concentrations give rise to cell death, as seen from disintegrated nuclei and a reduction in actin cytoskeleton labelling.

These preliminary results suggest that incorporation of DMS instead of S does not significantly alter SMA-HYD polymers' toxicity, which remains relatively low, making them in principle suitable for the mentioned applications, where biocompatibility is required.

4. Conclusions

In the light of a greener and sustainable chemical industry, many efforts have been put into the use of biobased monomers as valid substitutes of oil-based monomers, such as styrene. Following this trend, in this study, the use of p,α-dimethylstyrene as a monomer potentially bioderived from limonene or p-cymene [14,15], was tested as valid substitute of styrene in the preparation of amphiphilic SMA-like copolymers.

Two different series of SMA and DMSMA characterized by different molecular weight were successfully synthesized via RAFT in this work. The synthesis of SMA polymers via RAFT is not new in literature [37], and also the introduction of various derivatives of α-methylstyrene in SMA-like polymers has already been extensively studied from a synthetic point of view [23], although not including the potentially biobased p,α-dimethylstyrene studied here.

The main novelty and focus of this work was in the study of solution properties of such polymers, as a function of their structure, to investigate if the replacement of styrene with its alternative would have dramatic effects.

Interesting results can be found when the solution properties of those polymers are investigated. It was observed that both SMA-HYD and DMSMA-HYD are not able to form interpolymer aggregates when they are in solutions, as confirmed by DLS and cryo-TEM measurements. However, as evidenced by fluorescent probe experiments, hydrophobic domains are present, in line with the polyelectrolyte theory of alternating copolymers [56]. Furthermore, interesting results are highlighted by surfactant properties studies. DMSMA-HYD copolymers seemed slightly more surface-active than SMA-HYD when the M_n was kept constant. This could be described to the higher hydrophobicity of DMS compared S [70,71], and to the different dispersity ($Đ$). The latter seems to be confirmed by the fact that the DMSMA-HYD FR, having a much higher value of $Đ$, resulted in being the polymer with a lower surface tension value overall. No differences were observed in the use of S or DMS on the Dynamic Surface tension of those polymers. The latter one seems to depend only on the M_n of the polymer, as expected.

Finally, preliminary cytotoxicity tests show that replacing S with DMS does not seem to alter toxicity significantly. Those results bode well in the perspective of using DMSMA-HYD as an alternative to SMA-HYD as polymeric surfactants for various applications and can represent a good starting point for further investigations. Future work on these systems should include a study of the influence of pH and salinity on solution properties, emulsification and dispersion experiments to test our polymers for practical applications, and biodegradability test to assess their sustainability.

Author contributions

The manuscript was written through the contributions of all authors. N.M.: experimental work (except toxicity tests), data curation, writing – main author; T.v.K.: experimental work (toxicity tests), writing of the corresponding text; G.R.: conceptualization, manuscript revision; F.P. manuscript revision; P.R.*: conceptualization, supervision, project management, manuscript revision. All authors have approved the final version of the manuscript.

Data availability

Raw data are available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reactfunctpolym.2022.105204.

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