Polymer Testing 94 (2021) 107046

Contents lists available at ScienceDirect

Polymer Testing

journal homepage: http://www.elsevier.com/locate/polytest

Preparation, thermoresponsive behavior, and preliminary biological study of functionalized poly(*N*-isopropylacrylamide-*co*-dopamine methacrylamide) copolymers with an organotin(IV) compound

Alberto García-Peñas [a,b,c,*], Yu Wang [a], Irene Mena-Palomo [d], Eduardo López-Collazo [d], Diana Díaz-García [e], Santiago Gómez-Ruiz [e], Florian J. Stadler [a]

[a] College of Materials Science and Engineering, Shenzhen Key Laboratory of Polymer Science and Technology, Guangdong Research Center for Interfacial Engineering of Functional Materials, Nanshan District Key Laboratory for Biopolymers and Safety Evaluation, Shenzhen University, Shenzhen, 518055, PR China
[b] Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen, 518060, PR China
[c] Current Address: Departamento de Ciencia e Ingeniería de Materiales e Ingeniería Química (IAAB), Universidad Carlos III de Madrid, 28911, Leganés, Madrid, Spain
[d] Innate Immunity Group, Laboratory of Tumour Immunology, IdiPAZ Institute for Health Research, La Paz University Hospital, Madrid, 28046, Spain
[e] COMET-NANO Group, Departamento de Biología y Geología, Física y Química Inorgánica, ESCET, Universidad Rey Juan Carlos, Calle Tulipán S/n, 28933, Móstoles, (Madrid), Spain

ARTICLE INFO

Keywords:
Thermoresponsive polymers
Organotin(IV) derivatives
Functionalized structures
Biocompatibility

ABSTRACT

Recent advances focused on smart polymers have demonstrated the numerous advantages regarding other structures because they can adapt the behavior depending on physicochemical properties. In this way, functionalized thermoresponsive polymers with organometallic complexes were profoundly analyzed. Consequently, novel catalytic systems or biomedical devices could be developed. This publication focuses on the facile preparation of poly(*N*-isopropylacrylamide-*co*-dopamine methacrylamide) copolymers functionalized with triphenyltin chloride by protonolysis through the –OH of catechol groups. The presence of hydrophobic organotin(IV) derivatives could modify the solubility, thermoresponsive behavior, and other properties regarding pure copolymers. Also, sensitive analysis of the microstructure could help to understand the changes associated with the lower critical solution temperature by rheology, UV–vis spectroscopy, and calorimetry. In addition, a preliminary biological study against MDA-MB-231 cancer cells and peripheral blood mononuclear cells showed that the functionalized copolymers could be a potential platform to be explored in the future in the fight against cancer.

1. Introduction

The development of hybrid materials is continually growing due to the possibility of expanding the property portfolio of pure materials. In this context, the area of smart materials can offer additional features, as they can respond by themselves to external physicochemical stimuli [1,2]. These are sensitives to temperature, ions, pH, and can also provide reversibility, keeping good mechanical stability [3–6]. In particular, temperature-sensitive materials open exciting possibilities in biomedicine because non-invasive therapies can be achieved.

The addition of a second comonomer along polymeric chains of thermoresponsive structures is beneficial due to the introduction of hydrophilic or hydrophobic units that can be used to adjust the lower critical solution temperature (LCST) to specific necessities, adjusting the properties and developing new functionalities [7]. For instance, the use of catechol moieties, namely 3,4-dihydroxy phenyl-$_L$-alanine, leads to lower LCSTs and can provide reversible interactions with Fe^{3+} [8,9].

The fusion of polymers and inorganic fragments such as metals or metal-based compounds delivers new features and improves the response of pure polymers, and can be used as supported catalytic systems, among others [10–16]. In this context, the incorporation of organometallic complexes in polymeric structures could be suitable through functionalization to get well-defined structures for advanced catalytic systems or biomedical devices. Nevertheless, some

* Corresponding author. College of Materials Science and Engineering, Shenzhen Key Laboratory of Polymer Science and Technology, Guangdong Research Center for Interfacial Engineering of Functional Materials, Nanshan District Key Laboratory for Biopolymers and Safety Evaluation, Shenzhen University, Shenzhen, 518055, PR China.
E-mail addresses: alberto.garcia.penas@uc3m.es (A. García-Peñas), santiago.gomez@urjc.es (S. Gómez-Ruiz), fjstadler@szu.edu.cn (F.J. Stadler).

https://doi.org/10.1016/j.polymertesting.2020.107046
Received 19 November 2020; Received in revised form 22 December 2020; Accepted 29 December 2020
Available online 31 December 2020

shortcomings should be considered as organometallic compounds that are very sensitive to water and oxygen [17 19].

There are relatively few works about combining the thermosensitive polymers and organometallic complexes, and a significant part is focused on poly(*N*-isopropyl acrylamide) (PNIPAM) homopolymers and some derivatives [20,21]. The use of PNIPAM as a framework of new functionalized structures can offer new applications for drug delivery [22], catalytic systems [21], or nanotechnology [23]. These new systems can answer multiple demands from green chemistry, due to its ability to reduce organic solvents and efficiency [21]. Furthermore, the reversible thermoresponsiveness offered by these polymers could be a suitable option for many applications, in terms of recycling and regeneration. Nevertheless, the preparation of these hybrid materials usually carries out long and complicated procedures that involve numerous steps during the synthesis procedure or post-polymerization reactions. In addition, the use of additional reactants, solvents or conditions increases the costs and time of operation. These difficulties reduce the efficiency of the procedure in terms of a sustainable development. For that reason, new ways and methods for developing these new structures need to be addressed.

In this way, we propose the facile functionalization of the OH groups of hydrophobic dopamine methacrylamide randomly inserted along polymeric chains of N-isopropylacrylamide with organometallic complexes. This easy post-polymerization procedure could generate a new family of thermoresponsive materials with additional properties, especially useful for biomedicine or catalytic systems.

This work focuses on preparing functionalized poly(*N*-isopropylacrylamide-*co*-dopamine methacrylamide) copolymers with triphenyltin moieties, which come from the protonolyis reaction between copolymer and triphenyltin chloride (Ph_3SnCl). Also, some reactions were carried out in the presence of triethylamine. The molecular features of the resulting hybrid materials were deeply studied by FTIR-spectroscopy, proton nuclear magnetic resonance, gel permeation chromatography, and UV vis spectroscopy. The phase transition temperature was checked by UV vis spectroscopy, differential scanning calorimetry, and rheology; differences were tested according to DMA-content, end-group effect, molar mass, and the presence of triphenyltin moieties. Finally, the potential biological applications of this kind of hybrid materials were studied through different tests. The biocompatibility *in vitro* against cells of the immune system was analyzed, and the cytotoxic anticancer properties of the hybrid systems were tested against a highly resistant cancer cell line such as MDA-MB-231.

2. Materials

Methacrylic anhydride (94%, Macklin, Shanghai, China), sodium tetraborate decahydrate (99%, Macklin, Shanghai, China), sodium hydrogen carbonate (99.8% Macklin, Shanghai, China), 3,4-dihydroxy phenethylamine hydrochloride (98.5%, Aldrich, Saint Louis, USA), $MgSO_4$ (Aladdin, 99.9%, Shanghai, China), ethyl acetate (Hushi, Shanghai, China), chlorotriphenylstannane (Ph_3SnCl, 96%, Aldrich, Tianjin, China), N,N-diethylethanamine (NEt_3, 99%, Macklin, Shanghai, China), NaOH (96%, Aldrich, Shanghai, China), HCl (1 N, Macklin, Shanghai, China), chloromethylbenzene (99%, Macklin, Shanghai, China), methanedithione (98%, Aladdin, Shanghai, China), n-hexane (97%, Aladdin, Shanghai, China), toluene (99.5%, Hushi, Shanghai, China), dimethyl sulfoxide-d_6 (DMSO-d_6, 99.9%, Macklin, Shanghai, China), diethyl ether (99.5%, Lingfeng, Shanghai, China), tetrachloromethane (98%, Macklin, Shanghai, China), *p*-toluenesulfonic acid (99%, Macklin, Shanghai, China), and styrene (99.5%, Macklin, Shanghai, China), were purchased and used without pretreatment.

2,2 -Azobis(2-methylpropionitrile) (AIBN, 98%, Aldrich, Saint Louis, USA) and *N*-isopropyl acrylamide (NIPAM, 98%, Xiya Reagent, Shandong, China) were purified by recrystallization. *N,N*-dimethylformamide (DMF, 99.9%, Macklin, Shanghai, China) and tetrahydrofuran (THF, 99.9%, Aladdin, Shanghai, China) were treated before used as solvents. The purified water was obtained by a water purification system (Elix essential 5, Merck Millipore, Molsheim, France). The RAFT-agent, 1-phenyl ethyl phenyl dithioacetate (PEPD), was prepared following the procedure reported in the literature [24]. The obtained PEPD was purified by passing through a chromatography column.

2.1. Preparation of dopamine methacrylamide (DMA)

The comonomer dopamine methacrylamide (DMA) was synthetized as it was previously reported [25]. Furthermore, the DMA was purified by precipitation in hexane.

2.2. Synthesis of poly(N-isopropylacrylamide-co-dopamine methacrylamide) copolymers

The poly(*N*-isopropylacrylamide-*co*-dopamine methacrylamide) copolymers were prepared under inert conditions. First, both *N*-isopropylacrylamide (NIPAM) and DMA were placed inside of Schlenk tubes. At that point, the initiator (AIBN) and the RAFT agent (PEPD) were added using different amounts, as Table 1 shows. Finally, an amount of 10 mL of solvent (DMF) was added, keeping the inert atmosphere of nitrogen. The polymerizations took place in a thermostatic bath at 70 C for 48 h. The polymers were precipitated in diethyl ether, purified, dried, and stored at room temperature, as was previously carried out by this research lab [7].

The samples were denominated using a single C, followed by a number, as can be deduced from Table 1. Furthermore, polymeric water solutions were prepared to analyze the thermoresponsive behavior of the obtained copolymers.

2.3. Functionalization of copolymers with triphenyltin chloride

The functionalization was carried out through protonolysis using Ph_3SnCl as an organometallic precursor and under inert conditions. The dried copolymers were placed inside the Schlenk tubes, and subsequently, 15 mL of dried THF was added. Afterward, the organometallic compound was incorporated into the polymeric solutions. Some reactions were also performed in the presence of NEt_3, as summarized in Table 2. The functionalization reactions were carried out under vigorous stirring for 24 h, keeping the inert atmosphere of nitrogen. Then, reactions performed in the presence of NEt_3 were filtered, and the solvent was evaporated for all cases.

The new structures were denominated like the normal copolymer, followed by Sn, as a reference of the functionalized structure in the presence of Ph_3SnCl. Furthermore, the reactions performed using NEt_3 were also distinguished from normal functionalized materials by NEt_3.

2.4. Molecular characterization

The molar mass and polydispersity were studied using size exclusion chromatography (Beijing Wenfen LC98IIRI, Beijing, China), incorporating two polystyrene gel columns (Shodex, KD-803, and KD-806, Detector: RI-201H). The analysis was carried out using THF as a solvent (1 mL/min) at 40 C. The instrument was previously calibrated by narrow molecular mass distribution of polystyrene standards.

The final structure was checked by Fourier transform infrared spectrometer (Nicolet 6700, Waltham, USA), using tablets where the samples were embedded in potassium bromide. For that purpose, 32 scans were

Table 1
Monomers, initiator, and RAFT-agent amounts, used for polymerizations.

Name	NIPAM(mol)	DMA(mol)	AIBN(mol)	PEPD(mol)
C1	0.0177	0.00106	6.09 10 ^{6}	5.91 10 ^{5}
C2	0.0177	0.00106	9.13 10 ^{6}	1.18 10 ^{4}
C3	0.0177	0.00018	6.09 10 ^{6}	5.60 10 ^{5}

Table 2
Reactant amounts involved in the functionalization process.

Name	Ph_3SnCl (mol)	NEt_3 (mol)
C1 Sn	1.15 10 ^{6}	
C1 Sn NEt_3	1.15 10 ^{6}	1.15 10 ^{6}
C2 Sn	9.08 10 ^{7}	
C2 Sn NEt_3	9.08 10 ^{7}	9.08 10 ^{7}
C3 Sn	7.39 10 ^{8}	
C3 Sn NEt_3	7.39 10 ^{8}	7.39 10 ^{7}

performed per experiment with a resolution of 4 cm ^{1}. This analysis was also performed by proton nuclear resonance (AVANCE III 600 MHz spectrometer, Bruker, Basel, Switzerland), which can also provide information about the final composition of the polymeric chains. The samples were dissolved in DMSO-d_6, and a temperature of 25 C was selected for these experiments.

The incorporation of the organometallic fragment along the copolymers was analyzed using an X-ray fluorescence spectrometer (S4-Explorer, Bruker, Basel, Switzerland), which could provide valuable information about the amount of Sn in the samples.

2.5. Thermoresponsive behavior

The thermoresponsive behavior was studied for the new samples, which were previously dissolved in water (2 wt%) and stored in a refrigerator for 12 h. That time was considered enough for the homogenization of the samples. The phase transition temperature, associated with the LCST, was studied by UV vis spectroscopy, differential scanning calorimetry, and rheology.

The analysis performed in a UV vis spectroscopy (Lambda 365, PerkinElmer, Seoul, Korea) was carried out using a heating rate of 1 C/min at 400 nm. The range of temperatures of this study was selected between 5 C and 40 C.

The samples were sealed in aluminum pans for the analysis by differential scanning calorimetry (Q200, TA Instruments, New Castle, PA, USA), incorporating a cooling system.The experiments were carried out under nitrogen atmosphere using a flow of 40 mL/min. The final weight of the samples was around 5 mg, and the experiments were performed between 0 and 50 C, using a heating rate of 5 C/min. The results were normalized by another experiment performed for pure water under the same conditions.

Finally, the LCST was also tested by rheology (Anton Paar MCR 302 rheometer, Graz, Austria), using a cone-plate geometry (25 mm/1). The experiments were carried out in the linear viscoelastic regime (q 1 K min ^{1}, 1 rad/s, and $_0$ 0.2%), preserving the humidity of the samples in a saturated atmosphere.

All experiments were repeated at least three times, and in all cases, consistent results were obtained.

2.6. Cytotoxicity studies

2.6.1. Monocyte and lymphocyte isolation from peripheral blood and cell culture

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy donors by centrifugation on the Ficoll-Plus gradient (GE Healthcare Bio-Sciences), as reported previously [26,27]. PMBCs were cultured for at least 1 h at an initial density of 300 cells/mL in Roswell Park Memorial Institute (RPMI) medium (Invitrogen) supplemented with antibiotics (100 IU/mL penicillin and 100 lg/mL streptomycin). After this period, the supernatant was removed, and the adherent cells were cultured in the same medium supplemented with antibiotics (100 IU/mL penicillin and 100 lg/mL streptomycin) and 10% heat-inactivated normal serum (FBS) and were maintained overnight. The next day, different concentrations (20, 40, 50, 200, and 400 g/mL) of polymeric materials dissolved in sterile DMSO/PBS were added and incubated for 24 h. After this period, adherent cells were washed in 1X Phosphate Buffered Saline (PBS) and were removed to cytotoxicity analysis.

The human breast epithelial cell line MDA-MB-231 (a triple-negative receptor cell line) was grown in Dulbecco s-modified Eagle s medium (DMEM, Gibco) supplemented with antibiotics (100 IU/mL penicillin and 100 lg/mL streptomycin) and 10% heat-inactivated fetal bovine serum (FBS, Gibco). Cells were propagated and cultured at 37 C in a 5% CO_2 atmosphere. For treatment with polymeric materials, cells were counted and plated an initial density of 300 cells/ml overnight. After this period, cells were treated with the indicated concentrations of polymeric materials dissolved in sterile DMSO and were incubated for 24 h. Then, adherent cells were washed in 1X Phosphate Buffered Saline (PBS) and were removed to cytotoxicity analysis.

2.6.2. Flow cytometry assays and data analysis

For cytotoxicity assays, cells were labeled with phycoerythrin (PE)-conjugated propidium iodide (PI) from Invitrogen. Unstained cells were used as negative controls. The cells were incubated for 1 min at 4 C in the dark. The data were acquired by flow cytometry using a BD FACS-Calibur flow cytometer (BD Biosciences) and analyzed with FlowJo vX.0.7 (FlowJo, LLC) software and Prism 8.0 (GraphPad) software.

3. Results and discussion

The molecular characterization of the obtained copolymers was carried out before starting the functionalization. The molecular features will define the final properties of the new structures, especially the thermoresponsive behavior. The presence of organometallic moieties could affect these properties and could induce changes in the structure; consequently, a thorough evaluation of these characteristics will allow understanding the contributions of triphenyltin moieties.

First, the molar mass was analyzed for all copolymers by gel permeation chromatography. The GPC-curves, showed in Fig. 1a, exhibit a narrow molecular mass distribution whose polydispersity values (PDI) are pretty similar, around 1.3, with molar massess in the range of 9000 6000 g/mol such as shown in Fig. 1a. The polydispersity data achieved values close to unity, considering that reactions were performed using RAFT polymerization.

The structure was analyzed by Fourier-transform infrared spectroscopy, where differences in the structure cannot be observed, and exclusively changes must be attached to the comonomer composition, as Fig. 1b shows. In this context, other kinds of changes are not expected because the synthetic procedure, conditions, and reactants used during the preparation were the same, except for the monomer feed, which was varied systematically in the study. Understandably, the ratio defined between RAFT agent and initiator was adapted for that purpose.

Further information can be obtained through proton nuclear magnetic resonance, where the structure and composition of the polymeric chains can be analyzed. The ^{1}H NMR spectra were collected using deuterated DMSO-d_6, whose signal is identified at 2.5 ppm, as shown in Fig. 1c. The copolymers are statistical, as concluded from the protons positions, as previously published by our group [1,7]. The composition of the comonomers, NIPAM, and DMA can also be determined by the ^{1}H NMR spectrum, using the specific protons exhibited in Fig. 1d. In this case, the methine proton of NIPAM ($Area_{NIPAM}$) placed at 3.8 ppm and the benzene ring protons of DMA ($Area_{DMA}$) located around 6.7 ppm, were integrated and compared to achieve the composition using the following equation:

where [DMA] is the comonomer content (mol %), $Area_{NIPAM}$ is the integral of CH in NIPAM, and $Area_{DMA}$ the integral of the benzene ring protons of DMA. All the contents are displayed in Table 3, where similar

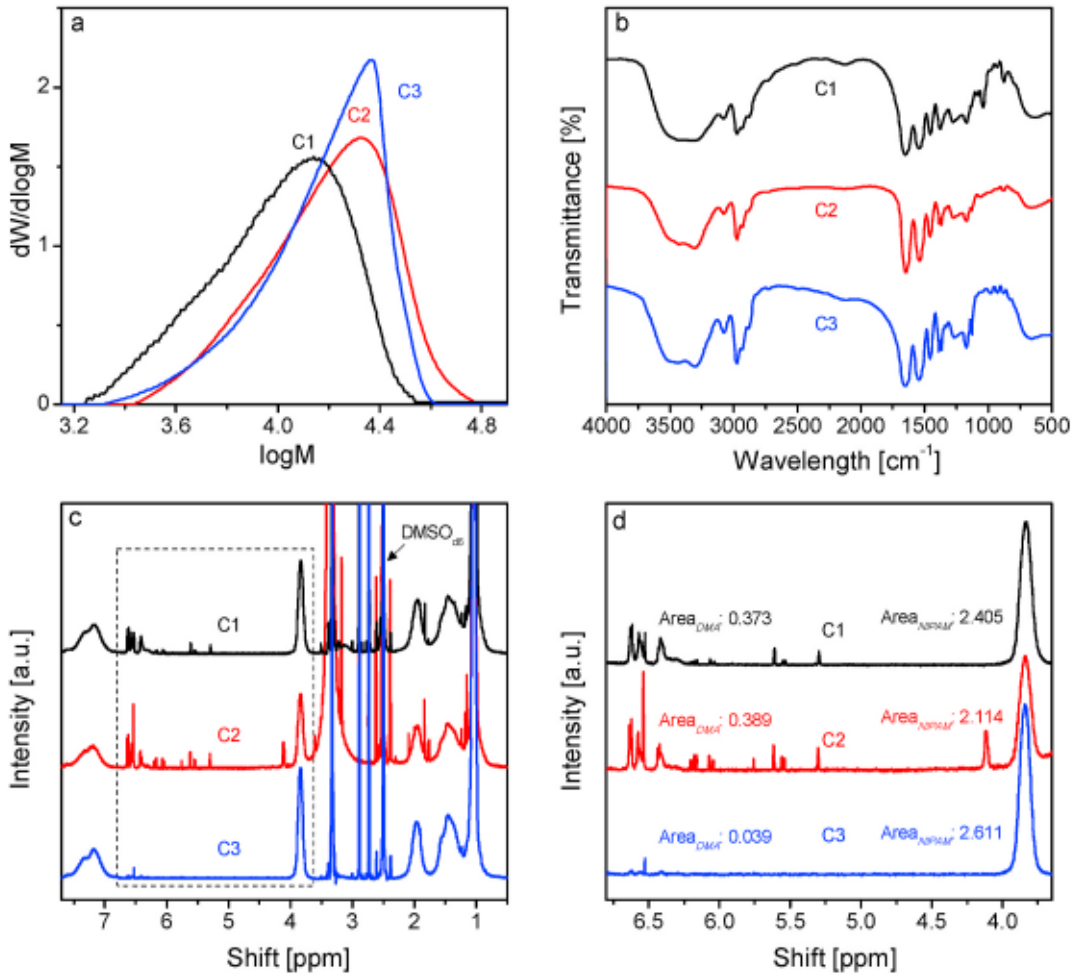


Fig. 1. The molar mass distributions (a), FTIR-spectra (b), and proton nuclear magnetic resonance (c, d) of random copolymers.

Table 3
Yield, comonomer content, molar mass, and polydispersity of random copolymers.

Name	Yield %	DMA-content mol %	Molar mass g/mol	Polydispersity
C1	40	5.7	9000	1.38
C2	75	6.1	13500	1.26
C3	60	0.5	13600	1.26

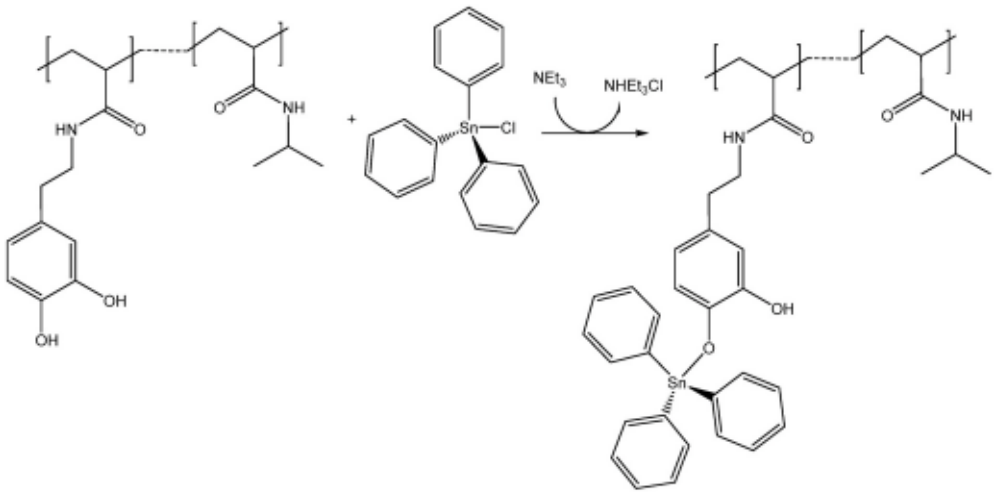
values are obtained for C1 and C2 compared to C3.

The protonolysis was carried out for all these copolymers following a similar procedure described previously by our group [28]. Scheme 1 shows the synthetic pathway, where conditions were kept constant except for the presence of NEt_3.

The loading of the copolymers with triphenyltin chloride, Ph_3SnCl, allows the formation of a sigma bond and the corresponding tin alkoxide compound (Scheme 1) incorporating the $SnPh_3$ moiety onto the polymer. The reaction was studied using a minimum amount of metal precursor for different reasons. First, this is a preliminary work where the presence of high content of triphenyltin moiety inside the polymeric chains could disrupt the structure. Second, this fact could alter the water solubility of the polymer, and subsequently, its thermoresponsive behavior. In addition, the incorporation of two Ph_3SnCl units in a single DMA-unit through both –OH groups can be restricted by the steric hindrance. Thus, the feeding of Ph_3SnCl was minimized (1.5%) to observe the influence of these organometallic moieties in the properties of the polymer while trying to preserve its solubility in water, and the thermoresponsive behavior.

The analysis of the loading of Ph_3SnCl was carried out through diverse methods such as X-ray fluorescence (XRF), which provides essential information about the content of Sn-linked to the polymeric structure. XRF is a quantitative analysis, but data obtained were close to the detection limits of this technique (defined around 0.02%). The materials showed values between 0.03 and 0.07% of Sn (Table 4), which confirm the presence of Sn inside the polymeric structure. Even though the quantities are very low, the results suggest that the protonolysis reactions work better in the presence of NEt_3, confirming the slight acidity of the protons of the catechol fragment.

The success of the functionalization was also investigated by proton nuclear magnetic resonance, FTIR-spectroscopy, UV–vis spectroscopy, and X-ray powder diffraction for all new structures. As an example of these results, Fig. 2 exclusively shows the results associated with C2 due to other functionalized copolymers exhibit similar results, and essential differences between samples were not observed like our group previously reported [28]. Fig. 2a displays the ^1H NMR spectra of C2, Ph_3SnCl, and functionalized copolymers. After the post-modification of the polymeric structure, some changes can be detected and assigned to their organometallic compounds. Specifically, the peak placed at 4.2 ppm could be associated with one of the free acidic protons of the catechol ligand. This signal did not appear in the non-functionalized polymer. It appeared after functionalization because the chemical environment of the catechol fragment has changed after functionalization, presumably impeding a rapid exchange of the protons as an $SnPh_3$ fragment substitutes one of the hydroxyl groups. In addition, the incorporation of the organometallic moiety is also observed with the appearance of very low-intensity peaks at ca. 7.4 and 7.8 ppm. These are associated with the



Scheme 1. Functionalization reaction between poly(*N*-isopropylacrylamide-*co*-dopamine methacrylamide) copolymer and the organometallic compound.

Table 4
Content of Sn of the final product measured by XRF.

Material	Sn (wt.%) final[a]
C1–Sn	0.039
C1–Sn–NEt_3	0.071
C2–Sn	0.056
C2–Sn–NEt_3	0.063
C3–Sn	0.054
C3–Sn–NEt_3	0.064

[a] Experimental Sn-content determined by X-ray fluorescence.

protons of the phenyl groups attached to tin [29]. The data obtained by FTIR-spectroscopy were displayed in Fig. 2b, and significant structural differences were not observed, confirming that the introduction of very low amounts of the organotin(IV) moiety does not significantly alter the final structure. The data obtained by X-ray powder diffraction (Fig. 2d) support the low functionalization rate showing very similar diffractograms for all the new structures .

The evidence of the functionalization was achieved by analyzing the material water solutions (0.1 wt%) at room temperature by UV-spectroscopy. The pure copolymer exclusively exhibits the band of the C═O as Fig. 2c displays for C2. Nevertheless, the functionalized copolymers defined another transition around 230 nm (appearing as a low-intensity shoulder) associated with the triphenyltin moiety as previously was reported for other structures [30,31]. The data obtained by X-ray powder diffraction (Fig. 2d) support the low functionalization rate showing very similar diffractograms for all the new structures.

The thermoresponsive behavior was analyzed for all functionalized copolymers (in the presence and absence of NEt_3 in the synthetic method) and compared to the pure copolymers. The results obtained by UV-spectroscopy were collected and displayed in Fig. 3a, where the phase transition temperature is sensibly lower than the PNIPAM homopolymer, which is defined around 32 °C [32–34]. This behavior is expected due to the presence of DMA content, whose LCST-trend is clearly defined by the content of DMA-units. Higher hydrophobic DMA-content will promote lower phase transition temperatures [1,7]. After the tin loading, the LCST decreased around 3 °C. The functionalized copolymers, prepared under the presence of trimethylamine, exhibited a lower LCST than other functionalized structures due to a slightly higher concentration of the hydrophobic organometallic fragments. Furthermore, the incorporation of the organotin(IV) compound led to broader transitions, justifying the locally inhomogeneous introduction of the organometallic moieties along the polymer chains. These local inhomogeneities are due to the low concentration of the organometallic fragments, which exert a local influence on the hydrophilicity, leading to inhomogenous hydrophilicity along the polymer chain.

Conventional calorimetry was also a valuable tool for detecting the phase transition temperature of these thermoresponsive materials (Fig. 3b). The data seem to follow the previous trend defined by UV–vis spectroscopy (Fig. 3a). However, some differences can be observed in pure copolymers. The phase transition temperature of C2 is slightly higher than C1, and this effect cannot be associated with the comonomer content. Nevertheless, differences in the molar mass could be strong enough to promote the end group effect over comonomer content, and LCST could decrease. The hydrophobic interactions of the RAFT agent could rise due to low molar mass, i.e., end groups will increase if the length of the polymeric chains decreases for the same polymer concentration in water [7,35].

The intensity and width of the signal obtained by calorimetry could play another important role, where hydrophobic interactions could lead to broader transitions, and the intensity of the peaks could be minimized [7,35]. Then, C3 shows a strong signal, as could be expected from its lowest content of comonomer and relatively high molar mass. This effect can also be observed when the metal compound is incorporated along with the polymeric structure, as C3–*Sn* promotes broader transitions at slightly lower LCSTs. Nevertheless, the LCST does not decrease very much as comonomer content is very low.

The rheology can provide valuable information about the final microstructure of the materials. Fig. 4 shows the complex shear modulus regarding the temperature. The results exhibit similar phase transition temperatures for all the pure copolymers observed by UV–vis spectroscopy and calorimetry, but C1 displays two transitions. Similar behavior was previously reported by our research lab, indicating that sections of polymeric chains defined by pure NIPAM could show an independent LCST of other polymeric sections composed by NIPAM and DMA co-units whose LCST would be lower [1]. The situation is somewhat different when C2 is analyzed, and a single transition can be observed. If C1 and C2 are compared, the differences need to be related to their molar masses because comonomer contents are equivalents for both cases. The C2 sample exhibits a single transition that should be associated with the random sequences of NIPAM and DMA, i.e., there are no significant amounts of long pure NIPAM-sequences or even PNIPAM homopolymers in the sample. This was concluded from calculations of the number of comonomers in the chain. Although the differences in comonomer content of C1 and C2 are not very large, the differences in the molar mass account of a massive difference in the fraction of chains without and with only one DMA-moiety (C1: 4% (0 DMA per chain) and 13% (1 DMA per chain) vs. C2: 0.4% (0 DMA per chain) and 2% (1 DMA per

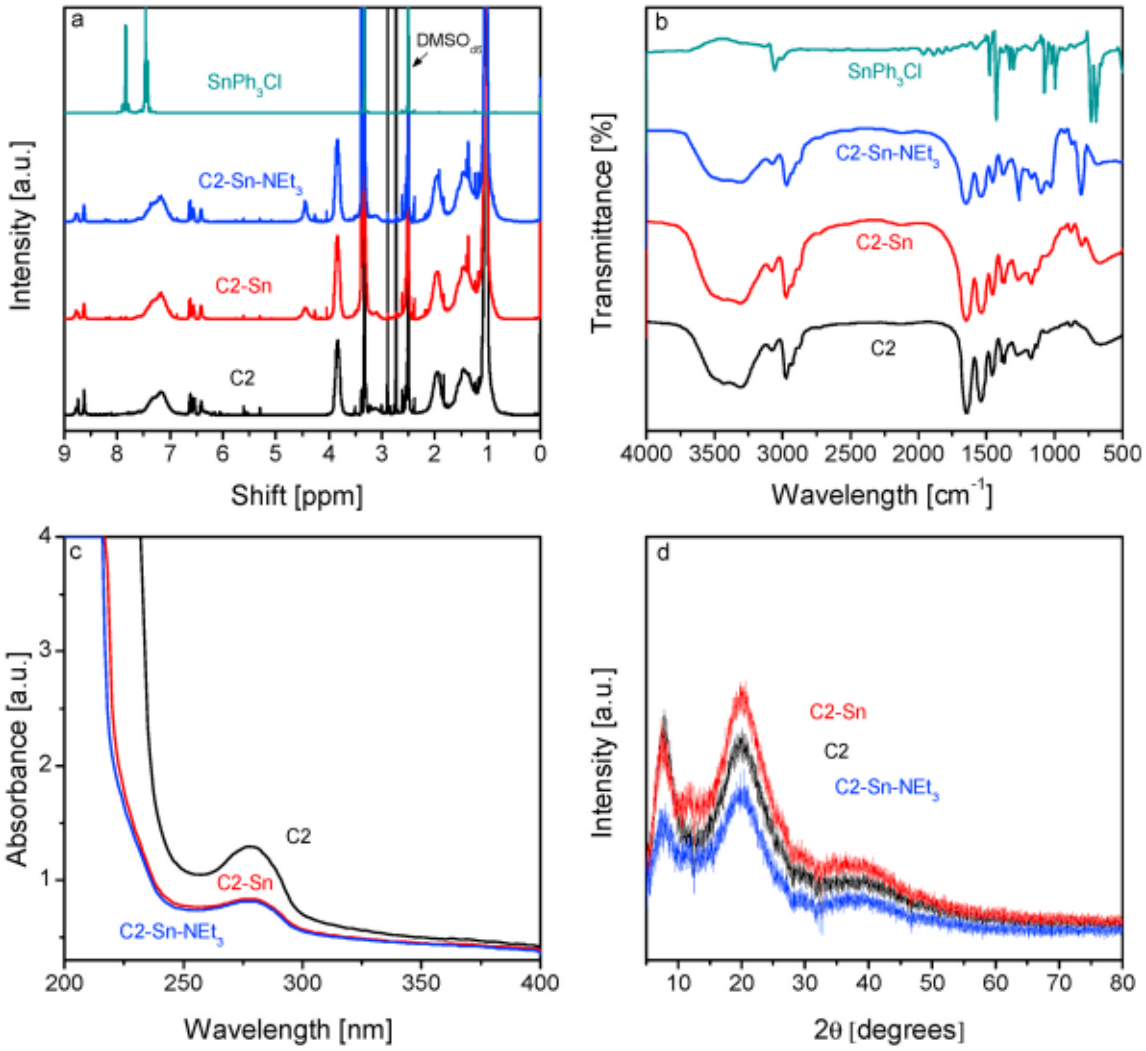


Fig. 2. Microstructure details of the functionalized copolymers obtained by proton nuclear magnetic resonance (a), FTIR-spectra (b), and UV–vis spectroscopy (c), and XRD (d).

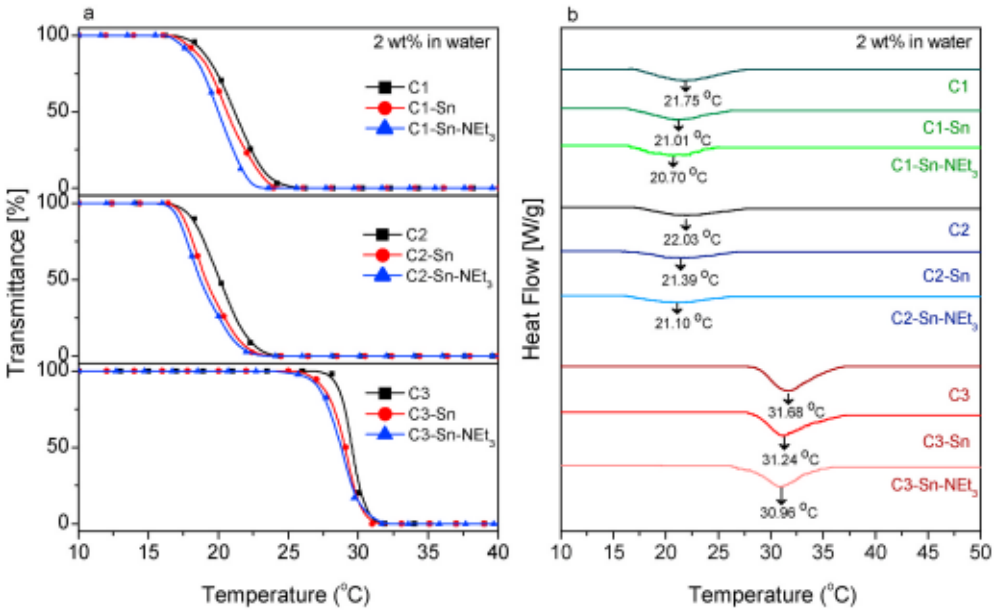


Fig. 3. Phase transition temperatures analyzed by UV–vis spectroscopy (a), conventional calorimetry (b).

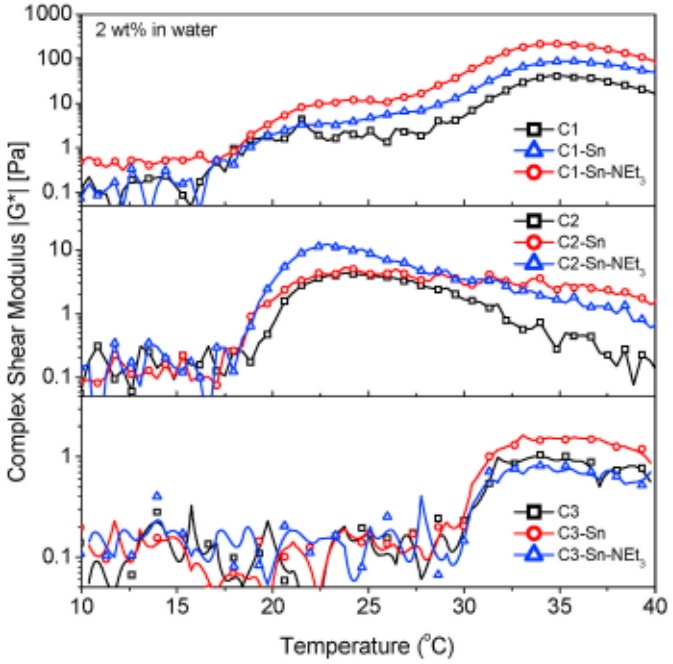


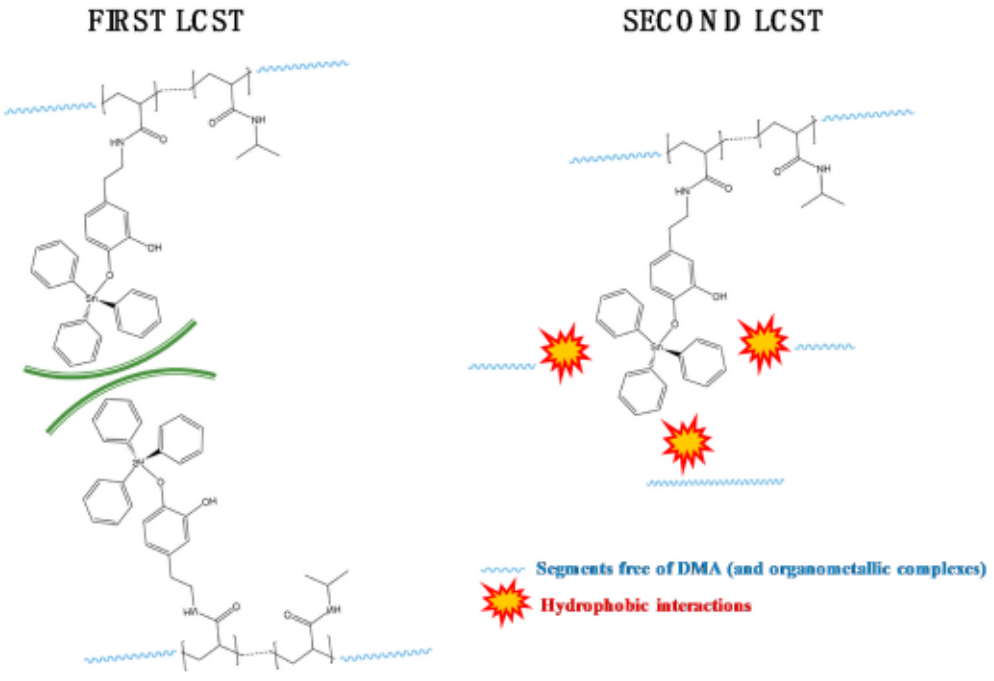
Fig. 4. Phase transition temperatures analyzed by rheology (c) for pure copolymers and functionalized copolymers.

chain) for $M = M_n$). Consequently, a small fraction PNIPAM-homopolymer in C1 causes the additional upturn with a maximum around 35 °C (typical of pure PNIPAM), as it delays the chain collapse until higher temperatures.

On the other hand, the organometallic moieties decrease the LCST, especially when the reactions were performed in the presence of NEt_3, probably due to a higher amount of organotin (IV) derivative loaded. C3, C3–Sn, and C3–Sn–NEt_3 show similar behavior due to the lowest amount of DMA-content, which does not allow for incorporating a higher amount of organometallic compound. The samples related to C2 show the highest difference between a pure copolymer and a functionalized system, due to the catechol groups considerably increase. The first LCST of C1 (Scheme 2) does not show differences between functionalized and non-functionalized copolymers because the hydrophobic interactions are directly associated with the hydrophobic DMA-units, which directly interact with the main backbone. Nevertheless, the introduction of the metal compound increases the hydrophobic forces between polymeric sections, which are free of DMA, and consequently, the second LCST decreases regarding the pure copolymer. The introduction of these complexes, through the –OH groups, rise the length of the catechol groups, increasing the possibilities to contact with other surrounding polymeric chains. Mainly, the interactions will take place with sections free of DMA, i.e., these could act as protection of the sections composed by NIPAM and NEAM monomers.

Fig. 5 collects all the phase transition temperatures estimated by UV-spectroscopy, calorimetry, and rheology. The approximations were carried out as described in previous reports of this research lab [35]. In general, the incorporation of an organometallic moiety tends to decrease the phase transition temperature of pure copolymers as can be deduced for all these materials and all the techniques explored. The influence of the comonomer content suddenly modifies the phase transition temperatures, showing a higher influence than the molar mass or the RAFT-agent, which are minimized [7,35]. The presence of the organometallic complex shows a small decrease in temperature, associated with its low content. The use of NEt_3 leads to load more organometallic complexes in the catechol groups, and consequently, the LCST decreases a little bit more.

An exception of the LCST-tendency can be observed for the lowest LCST of C1-samples observed through rheological measurements. This trend is opposite to the others, i.e., the LCST seems to rise with the presence of the organometallic compound. Nevertheless, this effect could be associated with the previous explanation, where the DMA-units and organometallic fragments could act as protection of the main backbone regarding surrounding polymeric chains. On the other hand, this finding could be associated with and artifact due to phase separation. Another explanation could be that the organometallic complexes interact with the stainless steel (Ni^{2+}, Fe^{3+}, Cr^{3+}, have been established to interact with DMA [36]) of the rheometer plates by adsorbing onto the plate surface or building complexes with ions released by the rheometer plates.



Scheme 2. Hypothetical schematic of hydrophobic interactions observed in functionalized C1, which shows two-phase transition temperatures.

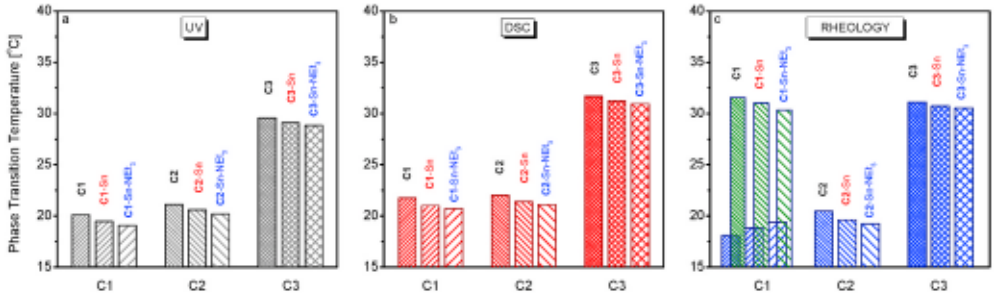


Fig. 5. Phase transition temperatures of the pure and functionalized copolymers estimated by UV–vis spectroscopy (a), calorimetry (b), and rheology (c).

After carrying out the complete characterization of the functionalized copolymers, a preliminary study of the potential biological application of these systems was performed in order to determine if, with an optimal functionalization of the polymers with a cytotoxic organometallic compound, they might become suitable for their future use in anticancer treatments. All the synthesized copolymers were tested *in vitro* against a cancer cell line of triple-negative breast cancer (MDA-MB-231, Fig. 6) and against immune cells (PBMC, Fig. 7) using concentrations between 20 and 400 μg/mL, which have demonstrated to be cytotoxic in previous studies of our group [37–39].

The preliminary results show that, generally, the non-functionalized copolymers are not active against any of the studied cells (cancerous and non-cancerous), which indicate a high degree of compatibility of these systems. However, when one analyzes the Sn-containing systems, it can easily be observed that the copolymers obtained by using NEt_3 as a base in the synthetic pathway (C1–Sn–NEt_3, C2–Sn–NEt_3, and C3–Sn–NEt_3) are always slightly more active than their corresponding analogs (synthesized without using NEt_3, namely C1–Sn, C2–Sn, and C3–Sn). It is important to note that, on a direct comparison of all the materials at low doses (between 20 and 200 μg/mL), the cytotoxicity against MDA-MB-231 is usually higher than that against PBMC (except in C2–Sn–NEt_3). This behavior indicates that, at low doses, these systems, modulated with a higher functionalization with the organotin(IV) cytotoxic compound, may become suitable for further anticancer studies, as they present a slight selectivity against cancer cell lines, compared with PBMC. However, the cytotoxicity of some of the systems (C1–Sn, C2–Sn,

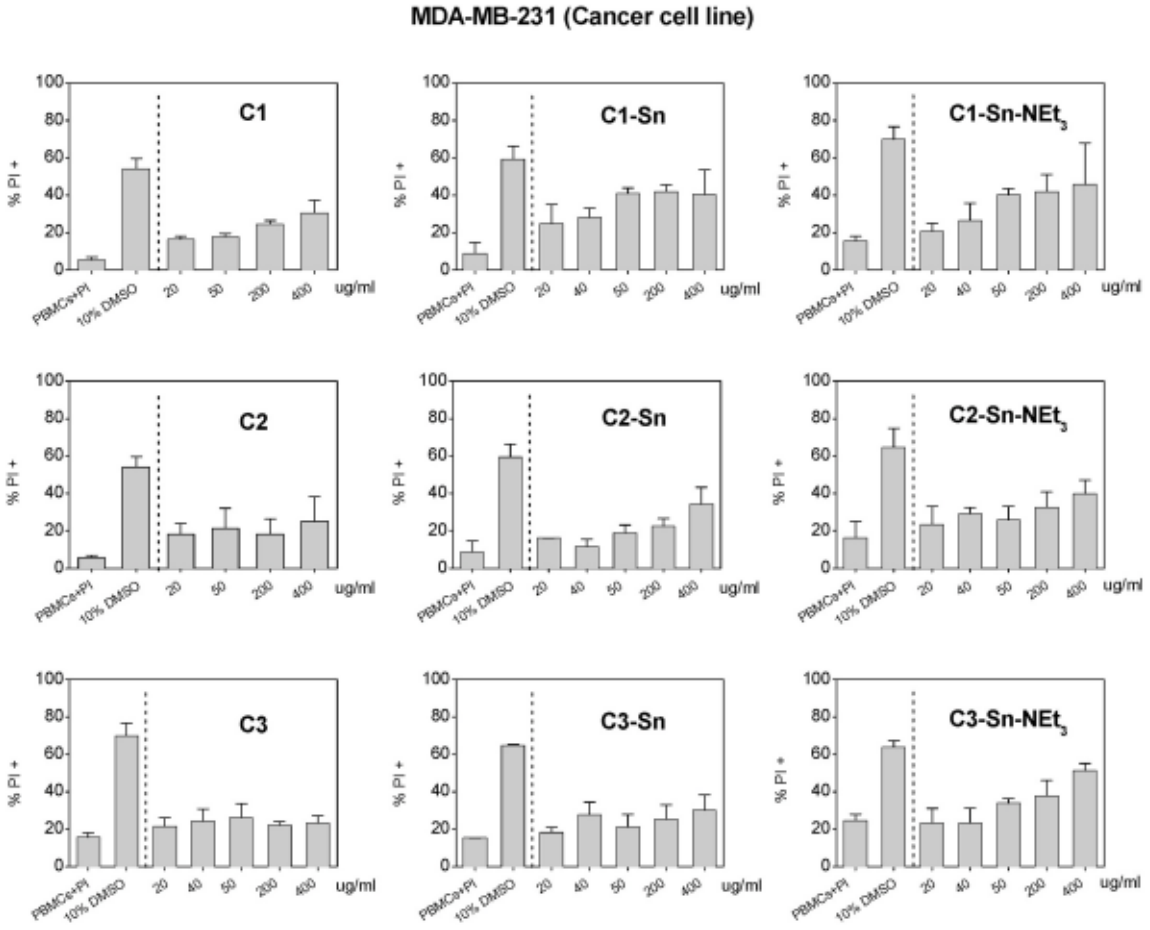


Fig. 6. Cytotoxicity assays for MDA-MB-231 in which cells treated with different amounts of copolymers were labeled with phycoerythrin (PE)-conjugated propidium iodide (PI). Unstained cells were used as negative controls, and the cells resulting from applying a solution of PBS and 10% DMSO as positive controls. The cells were incubated for 1 min at 4 °C in the dark, and the data were acquired by flow cytometry and analyzed with FlowJo vX.0.7 (FlowJo, LLC) software and Prism 8.0 (GraphPad) software. The higher the bar, the lower the cell survival.

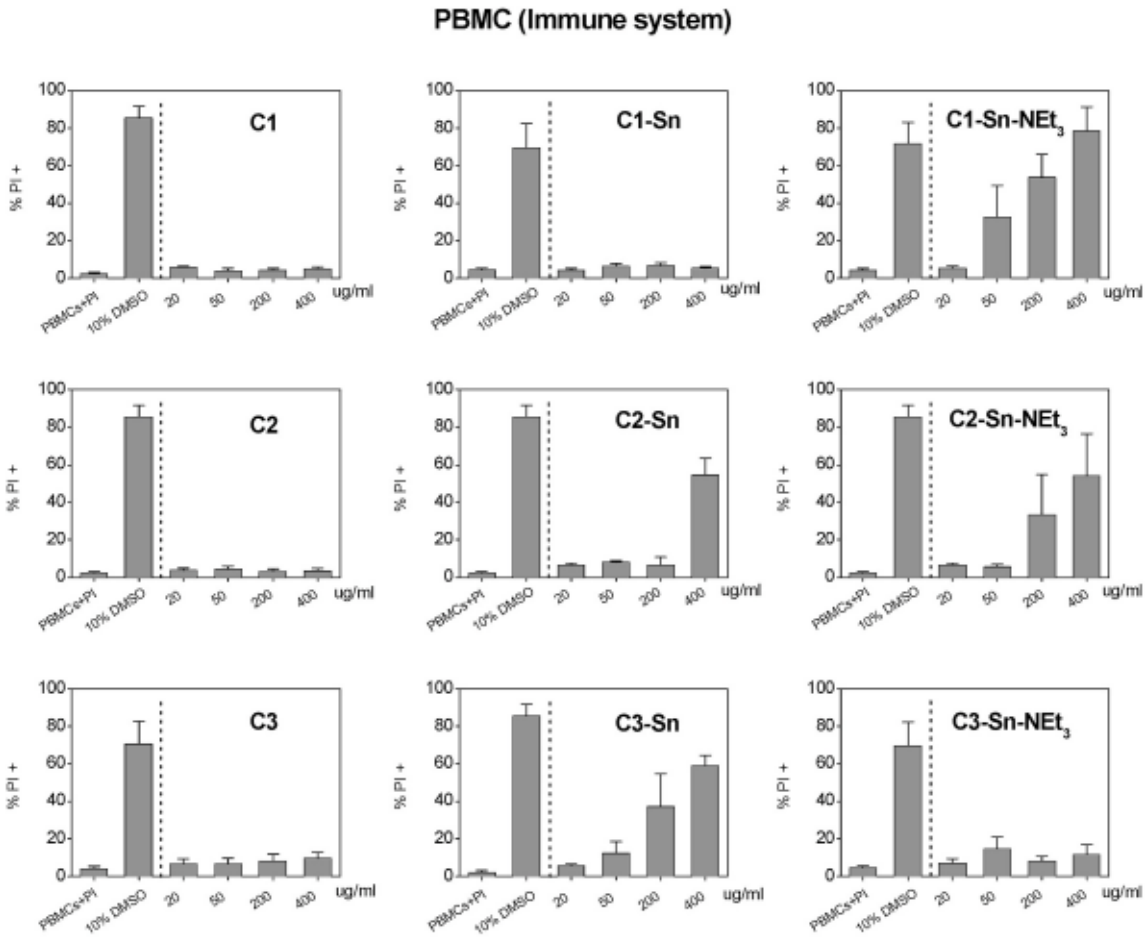


Fig. 7. Cytotoxicity assays for PBMC in which cells treated with different amounts of copolymers were labeled with phycoerythrin (PE)-conjugated propidium iodide (PI). Unstained cells were used as negative controls and the cells resulting of applying a solution of PBS and 10% DMSO as positive controls. The cells were incubated for 1 min at 4 °C in the dark and the data were acquired by flow cytometry and analyzed with FlowJo vX.0.7 (FlowJo, LLC) software and Prism 8.0 (GraphPad) software. The higher the bar, the lower the cell survival.

C1–Sn–NEt_3 and C2–Sn–NEt_3) at the highest tested concentration of 400 μg/mL is higher against PBMC than against MDA-MB-231, indicating a lower degree of applicability at these doses because of a low selectivity towards cancer cells.

Interestingly, the most attractive biological behavior has been found for C3–Sn and C3–Sn–NEt_3, which show a good degree of cytotoxicity against cancer cell lines MDA-MB-231 but do not affect the PBMC. These preliminary results validate further biological studies of these systems against some other cancer cell lines incorporating higher amounts of the cytotoxic compound. In addition, some studies should also be carried out using different temperatures to observe if the effect of the polymers and potential elimination to the medium of the tin compound may result in a thermo-responsive drug-delivery system for metallodrugs with potential applicability in chemotherapy.

4. Conclusions

The functionalized poly(*N*-isopropylacrylamide-*co*-dopamine methacrylamide) copolymers with the triphenyltin moiety were successfully obtained by protonolysis reactions as FTIR-spectroscopy, and ^{1}H NMR spectroscopy confirmed. In addition, the LCST-sensitivity of the calorimetry and UV–vis spectroscopy could allow the detection of the organometallic moieties because its incorporation leads to broader and more intense phase transitions.

The sensitivity of rheology can distinguish between the composition of polymeric sections and showing various LCST. Nevertheless, the molar mass and end-group effect will play an important role in the hydrophobic interactions over LCST. If the polymer molar mass is high enough and the comonomer content is sufficiently high, too, the formation of comonomer-free NIPAM-sequences is effectively suppressed, which leads to a single LCST. For lower molar masses, a small but non-negligible homopolymer fraction leads to a double LCST (lowest and highest LCST).

In addition, the surrounding hydrophobic interactions of polymeric chains over polymeric sequences enriched in comonomer content could be restricted if comonomer content is high enough, and consequently, the lowest LCST could remain constant. This effect could be improved if the organometallic complex is incorporated, avoiding hydrophobic interaction over those segments but promoting hydrophobic interaction on sequences of pure NIPAM, and consequently, the highest LCST could decrease.

Finally, a preliminary biological study of the functionalized copolymers against MDA-MB-231 cancer cells and PBMC showed that the functionalized copolymers C3–*Sn* and C3–*Sn*–NEt_3 presented a good degree of cytotoxicity against MDA-MB-231 cells, while not affecting the PBMC at low doses. These results will be validated in a more detailed future study of similar systems that, incorporating higher amounts of the cytotoxic tin compound and using different temperatures, may result in a thermo-responsive drug-delivery system for organotin compounds with potential application in chemotherapy.

CRediT authorship contribution statement

Alberto García-Peñas: Conceptualization, Methodology,

Supervision, Writing - original draft. **Yu Wang:** Visualization, Investigation. **Irene Mena-Palomo:** Investigation. **Eduardo Lopez-Collazo:** Visualization. **Diana Díaz-García:** Visualization, Investigation. **Santiago Gomez-Ruiz:** Supervision, Writing - original draft, Cytotoxicity studies, Data curation, Formal analysis. **Florian J. Stadler:** Supervision, Writing - review & editing.

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.

Acknowledgments

The financial support obtained from the National Science Foundation of China (21574086), Shenzhen Fundamental Research Funds (No. KC2014ZDZJ0001A), Shenzhen Sci & Tech research grant (ZDSYS201507141105130) and China Postdoctoral Science Foundation Grant (2018M633119) are acknowledged. Also, these researchers would like to thank the former Ministerio de Ciencia, Innovacion y Universidades of Spain (current Ministerio de Ciencia e Innovacion of Spain) for the grant RTI2018-094322-B-I00 and the Direccion General de Investigacion e Innovacion, Consejería de Educacion e Investigacion de la Comunidad de Madrid for the predoctoral grant PEJD-2017-PRE/BMD-3512 (I. M.-P.).

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