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Thiol-reactive (co)polymer scaffolds comprised of organic arsenical acrylamides

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Novel, well-defined organic arsenical homopolymers (*Đ* = 1.10 - 1.40) have been synthesised via RAFT polymerisation. Copolymerisation of the As-functional monomer with dimethylacrylamide yielded non-toxic polymer scaffolds (*Đ* ≈ 1.10) that could be manipulated in reponse to pH and undergo sequential reduction and substitution in the presence of thiols including cysteine and glutathione.

The concept of post-polymerisation modification[1] dates back to the 19th century and the beginning of polymer science. Processes such as vulcanisation, hydrogenation and acetylation of natural and synthetic polymers have been vital to the production and development of commodity materials. Over the last 20 years, the emergence and development of living/controlled radical polymerisation techniques has revolutionised the way in which polymers can be synthesised.[2-4] Through controlling the radical concentration via reversible deactivation or (degenerative) chain transfer mechanisms the degree of the polymerisation (DP_n) and molecular weight can be exquisitely controlled ranging from short oligomers to very high molecular weight polymers. Furthermore, the ability to retain high ω-chain end fidelity has been exploited to prepared (multi) block copolymer scaffolds, with exceptional precision, via sequential chain extension.[5, 6] Radical polymerisation techniques, particularly reversible addition fragmentation chain transfer polymerisation (RAFT), also benefit from broad functional group tolerance.[7, 8] This has allowed monomers with reactive pendent groups such as epoxides,[9] thiolactones,[10] azlactones,[11] activated esters,[12] aldehydes,[13] ketones,[14] pyridyl disulfide,[15] protected alkynes,[16] disubstituted maleimides,[17] *etc.*, to be employed to construct functional (co)polymer scaffolds that can be modified, post-polymerisation, using efficient and 'click'-type reactions. Through careful design, combining state-of-the-art polymerisation techniques with appropriate functional monomers can lead to complex polymer compositions that have complimentary or orthogonal functionalities. Such polymers have great potential in the biomedical medical field where orthogonal reactions can be applied to incorporate specific functionality and capabilities into complex polymer constructs in solution,[18] or polymer brushes grown from a surface.[19] For example, functionalisation of polymer chains with receptor specific ligands and therapeutics (active targeting),[20] imaging motifs and therapeutics (so called theranostics)[21] and multiple therapeutics (for combination therapies)[22] have all been investigated.

Recently, polymers synthesised with an organic arsenical at the α-chain end were shown to undergo efficient, highly selective reactions with thiol or disulfide bond containing proteins and peptides.[23] Arsenic can exist in two biologically relevant oxidation states, trivalent arsenous acid (As(III)) and pentavalent arsenic acid (As(V)) which are chemically distinct offering diverse possibilities for the development of responsive, reactive and dynamic polymers and materials. For example, As(V) does not form covalent bonds with thiols, preferring to undergo electron transfer processes, whereas As(III) has a high affinity for thiols and readily forms covalent bonds with unrivalled selectivity for thiols, particularly disulfide-derived dithiols, compared to thiol reactive maleimides.[23]

Although polymeric arsenicals have been synthesised previously, the focus was on the fundamental properties and applications of (co)polymers prepared by free radical

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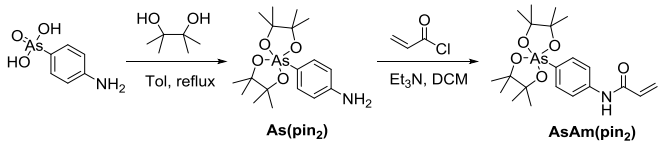
polymerisation (FRP).[24-28] However, to date, the distinctive reactivity of arsenicals has been overlooked with respect to incorporating such groups into functional polymer scaffolds. Herein, well-defined organic arsenical (co)polymer scaffolds have been prepared for the first time, using RAFT. Crucially, the reactive scaffolds have been shown to be non toxic and they can



Scheme 1. Synthesis of AsAm(pin$_2$) from *p*-arsanilic acid.

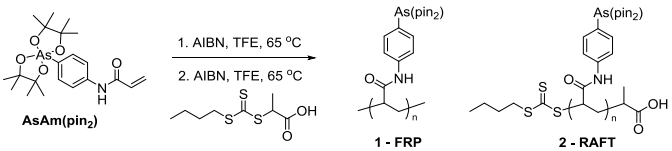
undergo a variety of transformations, post polymerisation. Model and functional thiols have been shown to sequentially reduce, and then undergo substitution at reactive arsenic centres.

Initially, a novel arsenic-containing (As(V)) monomer (AsAm(pin$_2$)) was synthesised in a two-step process from *p*-arsanilic acid to enable dissolution and polymerisation in organic media (Scheme 1). Synthesis of the arsenate (As(pin$_2$)) and AsAm(pin$_2$) was confirmed by ^{1}H NMR spectroscopy by the sequential appearance of the pinacol and vinyl group signals at 1.05-1.30 and 5.70-6.50 ppm, respectively (Fig. S1a). Further confirmation for the formation of As(pin$_2$) was obtained from IR spectroscopy (Fig. S1b) through which the broad O-H signals at 2000-3000 cm^{-1} were shown to disappear which coincided with appearance of C-O stretch at 1140 cm^{-1} and a small shift in the As-O peaks from 820 cm^{-1} to 875 cm^{-1} upon formation of the As(pin$_2$). Following amidation to form AsAm(pin$_2$), a signal corresponding to the amide carbonyl was also observed at 1690 cm^{-1}.

To determine if AsAm(pin$_2$) could undergo radical polymerisation, it was dissolved in 2,2,2-trifluoroethanol (0.5 M) and AIBN (1 mol%) was added prior to heating at 65 °C. The reaction was terminated after 330 minutes at 81 % monomer conversion, which was determined by comparing the disappearance of the vinyl peaks of AsAm(pin$_2$) at 5.70-6.50 ppm with the sum of the aromatic protons belonging to AsAm(pin$_2$) and the emerging polymer (PAsAm(pin$_2$)) at 7.30-8.50 ppm (Fig. S2a). Polymerisation was also possible in DMF, however solvent signals obscured accurate conversion analysis by ^{1}H NMR so TFE selected as the preferred solvent. Molecular weight analysis by SEC revealed the formation of the polymer with a broad molecular weight distribution characteristic of free radical polymerisation (M_n = 39000 g mol^{-1}, M_w = 97900 g mol^{-1}, $Đ$ = 2.51, Fig. S2b).

In an attempt to control the polymerisation of AsAm(pin$_2$), the reaction conditions were modified. 2-(((Butylthio)-carbonothioyl)thio)propanoic acid, a common chain transfer agent (CTA) for the RAFT polymerisation of acrylamides was added to target a degree of polymerisation (DP$_{n,th}$, [AsAm(pin$_2$)] / [CTA]) of 10. Pleasingly, a ^{1}H NMR spectrum taken after 310 minutes revealed 97 % conversion and the presence of a peak at 5.0 ppm corresponding to the trithiocarbonate group at the ω-chain end (Fig. S3). Furthermore, SEC analysis revealed good agreement between the theoretical ($M_{n,th}$) and experimental ($M_{n,SEC}$) molecular weight and a narrow molecular weight distribution (M_n = 4000 gmol^{-1}, $Đ$ = 1.10). The DP$_n$ was increased by increasing [AsAm(pin$_2$)] / [CTA] to 20 and 50. When targeting DP$_{n,th}$ = 20, 94 % conversion was achieved after 330 minutes, whilst the amount of initiator was increased ([CTA] / [I] = 5) for DP$_{n,th}$ = 50 to maintain a comparable rate of polymerisation (96%, 365 minutes). Though the molecular weight distributions were observed to increase to 1.15 and 1.38 respectively, they remained relatively narrow, indicative of good level of control over the polymerisation (Table 1, Fig. 1).

Table 1. Synthesis of PAsAm(pin$_2$) by RAFT and FRP.



[M]/ [CTA]	[CTA]/ [I]	t / min	Conv.	$M_{n,th}$ gmol^{-1}	$M_{n,SEC}$ gmol^{-1}	$Đ$
*	*	330	81%	*	39000	2.51
10	10	310	97%	4600	4000	1.10
20	10	330	94%	8600	5600	1.15
50	5	365	96%	22000	10000**	1.38

*FRP [M] = 0.5 M, [AIBN] = 1 mol%

**SEC$_{TD}$: $M_{n,TD}$ = 29500 gmol^{-1}, $Đ$ = 1.36, $R_{h,TD}$ = 2.5 nm, $R_{g,TD}$ = 3.0 nm, α = 0.44.

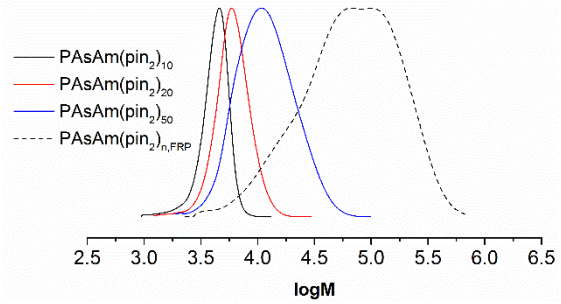


Fig 1. SEC (DMF) chromatograms of PAsAm(pin$_2$) synthesised by FRP and RAFT with increasing DP$_{n,th}$

Interestingly, increasing the DP$_{n,th}$ did not coincide with the expected increases in $M_{n,SEC}$. Thus, triple detection SEC (SEC$_{TD}$) was performed on a purified sample of PAsAm(pin$_2$) with DP$_{n,th}$ = 50 whereby combining the signals obtained from the DRI, viscometer and light scattering detectors resulting in a larger molecular weight and comparable dispersity being obtained ($M_{n,TD}$ = 29500 gmol^{-1}, $Đ$ = 1.36, Fig. S4). Furthermore, SEC$_{TD}$ revealed that the PAsAm(pin$_2$) adopted a random coil conformation in solution (DMF, $R_{h,TD}$ = 2.5 nm, $R_{g,TD}$ = 3.0 nm, α = 0.44).

Kinetic analysis of the polymerisation of AsAm(pin$_2$) (DP$_{n,th}$ = 50) was performed by periodic sampling of the polymerisation with complimentary ^{1}H NMR (Fig. S5) and SEC (Fig. S6) analysis. Pseudo first-order kinetics were observed (Fig. S7) and although $M_{n,SEC}$ tended to lower values than $M_{n,th}$ a linear evolution of molecular weight (Fig. S8) was nonetheless observed as expected for a controlled radical polymerisation. Further evidence to support the controlled nature of the polymerisation was obtained via chain extension of a PAsAm(pin$_2$)-macroCTA (DP$_{n,th}$ = 10) with dimethylacrylamide (DMA) with DP$_{n,th}$ = 90. The polymerisation reached 99 % conversion within 18 hours

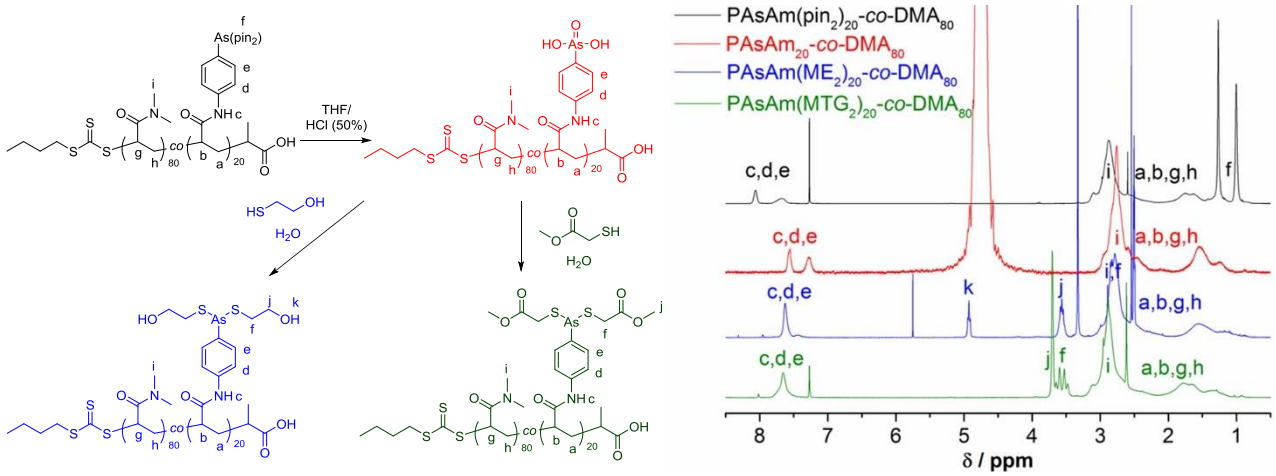


Fig 2. Manipulation of the polymeric arsenical scaffold by sequential treatment with acid and thiols confirmed by ^{1}H NMR, $CDCl_3$.

and successful chain extension was confirmed by ^{1}H NMR with the appearance of new signals corresponding to the methyl groups of DMA at 2.89 ppm and an enhancement in the polymer backbone signals at 1.5-2.5 ppm (Fig. S9a). Additionally, the signals related to AsAm(pin$_2$) and DMA exhibited a common diffusion coefficient in DOSY-NMR (Fig. S9b). Comparison of the integrals corresponding to AsAm(pin$_2$) (7.30-8.50 ppm) and DMA (2.89 ppm) revealed [AsAm(pin$_2$)] : [DMA] = [1] : [10] which was in good agreement with the initial monomer feed. The PAsAm(pin$_2$)$_{10}$-*b*-DMA$_{100}$ block copolymer retained a low dispersity (*Đ* = 1.10) with a complete shift in molecular weight observed in the SEC chromatogram of the purified polymer (Fig. S10). Direct dissolution of PAsAm(pin$_2$)$_{10}$-*b*-DMA$_{100}$ in D_2O resulted in self-assembly of the block copolymer into higher order aggregates as confirmed by ^{1}H NMR with broadening and disappearance of the hydrophobic aromatic and pinacol side chain signals due shielding by the well solvated DMA side chain (Fig S11). Aggregation was further supported by dynamic light scattering (DLS) revealing an average particle size of 147 nm (Fig S12).

Statistical copolymers PAsAm(pin$_2$)$_x$-*co*-DMA$_y$ with DP$_{n,x+y}$ = 100 and a monomer feed ratio (*x : y*) varying between 0 : 100 and 50 : 50 were synthesised as model As(V)-containing functional copolymer scaffolds (Table S2, Fig. S13). Monomer conversions were determined by comparing the decrease in distinguishable vinyl signals assigned to the AsAm(pin$_2$) (5.81 ppm) and DMA (5.72 ppm) with the sum of the emerging polymer and remaining monomer signals associated with each monomer (Fig. S14). Kinetic analysis of the polymerisation with a monomer feed ratio of *x : y* = 20 : 80 revealed that AsAm(pin$_2$) reacts at a faster rate than DMA, which is consistent with the observed final conversions at each monomer feed (Fig. S15, Table S2). A linear evolution of molecular weight ($M_{n,SEC}$) was observed when plot against the total monomer conversion (Fig. S16) and a symmetrical molecular weight distribution with low final dispersity (*Đ* = 1.09) were also obtained by SEC (Fig. S17).

Considering that the As(V)-functional group can undergo sequential reduction, to As(III), and substitution in the presence of excess thiol containing reagents,[29] it was postulated that in the presence of an excess of thiol per As(V) group, the As(V)-functional scaffolds would undergo modification yielding thiol-substituted As(III)-functional copolymers. For consistency the synthesis of PAsAm(pin$_2$)$_{20}$-*co*-DMA$_{80}$ was scaled up (M_n = 13200 gmol^{-1}, *Đ* = 1.09) and the pinacol groups were removed under acidic conditions to furnish the reactive PAsAm$_{20}$-*co*-DMA$_{80}$ scaffold (Fig 2). Mercaptoethanol (ME) and methyl thioglycolate (MTG) were selected as model thiols and added to aqueous solutions of PAsAm$_{20}$-*co*-DMA$_{80}$. Reduction and substitution of the pendent As-groups was confirmed via IR by the absence of the As-O stretches at 600-1000 cm^{-1} (Fig. S18a). Furthermore, ^{1}H NMR of both PAsAm(ME$_2$)$_{20}$-*co*-DMA$_{80}$ and PAsAm(MTG$_2$)$_{20}$-co-DMA$_{80}$ polymers (Fig. 2) revealed new signals corresponding to the substituted thiol groups. Signals at 4.93, 3.56 and 2.83 ppm were assigned to the pendent mercaptoethanol groups of PAsAm(ME$_2$)$_{20}$-*co*-DMA$_{80}$ and they shared a consistent diffusion coefficient with the signals ascribed to the copolymer backbone and remaining sidechains by DOSY-NMR (Fig. S18b). Integration of the signal at 3.56 ppm, assigned to the C*H*$_2$OH protons and comparison with the aromatic signals at 7.63 ppm revealed that all of the available As(V) groups had been successfully reduced and modified. New signals at 3.70 and 3.56 ppm in PAsAm(MTG$_2$)$_{20}$-*co*-DMA$_{80}$ were assigned to the pendent methyl and methylene groups present in the substituted methyl thioglycolate groups. Integration of these groups and comparison with the aromatic signal at 7.66 ppm again revealed full reduction and substitution of the pendent As-groups and DOSY-NMR revealed a single common diffusion coefficient (Fig. S18c). SEC analysis revealed that the post polymerisation modification process did not have a detrimental effect on the dispersity (*Đ* ≈ 1.25) of the modified polymers (Fig S18d).

The potential for dynamic covalent chemistry with these polymeric arsenicals was investigated by addition of an excess of thioglycerol to PAsAm(ME$_2$)$_{20}$-*co*-DMA$_{80}$. Complete substitution of the mercapoethanol by thioglycerol was confirmed by ^{1}H NMR, SEC and IR (Fig S19) suggesting that such polymers could have potential to respond to changes in thiol concentration and therefore redox environment, which could be adventitious for biomedical applications.

The $PAsAm_{20}$-*co*-DMA_{80} scaffold was then reacted with a thiol containing amino acid, cysteine ethyl ester hydrochloride (Cys) and tripeptide, glutathione (GSH). Pleasingly, the As-O stretches were again absent in the IR spectra (Fig. S20) and ^{1}H NMR revealed full reduction and substitution of the pendent As-groups furnishing $PAsAm(Cys_2)_{20}$-*co*-DMA_{80} and $PAsAm(GSH_2)_{20}$-*co*-DMA_{80} respectively (Fig. S21). $PAsAm(GSH_2)_{20}$-*co*-DMA_{80} was not soluble in DMF for SEC and although $PAsAm(Cys_2)_{20}$-*co*-DMA_{80} was soluble, SEC revealed a lower than expected molecular weight (M_n = 8900 gmol^{-1}, $M_{n,th}$ = 17600 gmol^{-1}, $Đ \approx 1.24$, Fig. S22) which suggests that the hydrodynamic volume of the modified copolymer differs significantly to the PMMA calibrants.

Considering the success of the polymeric arsenical scaffold synthesis, their subsequent modification and with potential biomedical applications in mind, evaluation of their toxicity is crucial. Thus, the acute toxicity of the $PAsAm_x$-*b*-DMA_y (co)polymers, the highest molecular weight homopolymer $PAsAm_{50}$ and the modified polymers was determined *in vitro* using a standard protocol for the XTT assay. Pleasingly, the $PAsAm_x$-*b*-DMA_y copolymers and $PAsAm_{50}$, containing As(V) pendant groups, were non-toxic up to 20 μM (Fig S23a) which is promising for their application as functional scaffolds. At higher concentrations (50 μM) the $PAsAm_{50}$ did exhibit greater toxicity compared to the $PAsAm_x$-*b*-DMA_y copolymers in the which addition of DMA dilutes the effective concentration of the arsenic group. Similarly, the modified copolymers, containing As(III) pendant groups, were also non-toxic with the exception of $PAsAm(MTG_2)_{20}$-*co*-DMA_{80} which showed viability up to 2 μM but a decrease in viability between 2 – 20 μM (Fig S23b).

In conclusion, polymeric arsenicals have been synthesised for the first time using a novel organic arsenical monomer. Well-defined (co)polymer scaffolds have been prepared via RAFT and can be manipulated under acidic conditions to furnish a reactive As(V) motif. Reactions with organic thiols as well as amino acids (cysteine ethyl ester hydrochloride) and peptides (glutathione), proceed by sequential reduction and substitution at the As-centre to yield modified polymer scaffolds. Crucially, the homo- and copolymer scaffolds were found to be non-toxic. Considering the discreet and distinctive reactivity of (in)organic arsenicals, it is envisaged that such scaffolds could lead to the development of a new class of responsive materials.

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References

1. K. A. Günay, P. Theato and H.-A. Klok, *J. Pol. Sci., Part A: Polym. Chem.*, 2013, **51**, 1-28.
2. W. A. Braunecker and K. Matyjaszewski, *Prog. Polym. Sci.*, 2007, **32**, 93-146.
3. G. Moad, E. Rizzardo and S. H. Thang, *Acc. Chem. Res.*, 2008, **41**, 1133-1142.
4. D. A. Shipp, *Polym. Rev.*, 2011, **51**, 99-103.
5. G. Gody, T. Maschmeyer, P. B. Zetterlund and S. Perrier, *Nat. Commun.*, 2013, **4**, 2505.
6. A. H. Soeriyadi, C. Boyer, F. Nyström, P. B. Zetterlund and M. R. Whittaker, *J. Am. Chem. Soc.*, 2011, **133**, 11128-11131.
7. D. J. Keddie, *Chem. Soc. Rev.*, 2014, **43**, 496-505.
8. G. Moad, *Polym. Chem.*, 2017, **8**, 177-219.
9. S. Edmondson and W. T. S. Huck, *J. Mater. Chem.*, 2004, **14**, 730-734.
10. S. Reinicke, P. Espeel, M. M. Stamenović and F. E. Du Prez, *ACS Macro Lett.*, 2013, **2**, 539-543.
11. D. C. Tully, M. J. Roberts, B. H. Geierstanger and R. B. Grubbs, *Macromolecules*, 2003, **36**, 4302-4308.
12. H.-G. Batz, G. Franzmann and H. Ringsdorf, *Angew. Chem. Int. Ed.*, 1972, **11**, 1103-1104.
13. G. Sun, C. Cheng and K. L. Wooley, *Macromolecules*, 2007, **40**, 793-795.
14. D. Rabuka, R. Parthasarathy, G. S. Lee, X. Chen, J. T. Groves and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2007, **129**, 5462-5471.
15. L. Wang, J. Kristensen and D. E. Ruffner, *Bioconjugate Chem.*, 1998, **9**, 749-757.
16. V. Ladmiral, G. Mantovani, G. J. Clarkson, S. Cauet, J. L. Irwin and D. M. Haddleton, *J. Am. Chem. Soc.*, 2006, **128**, 4823-4830.
17. Z. Tang, P. Wilson, K. Kempe, H. Chen and D. M. Haddleton, *ACS Macro Lett.*, 2016, **5**, 709-713.
18. Q. Zhang, A. Anastasaki, G.-Z. Li, A. J. Haddleton, P. Wilson and D. M. Haddleton, *Polym. Chem.*, 2014, **5**, 3876-3883.
19. J. O. Zoppe, N. C. Ataman, P. Mocny, J. Wang, J. Moraes and H.-A. Klok, *Chem. Rev.*, 2017, **117**, 1105-1318.
20. H. T. T. Duong, Z. Dong, L. Su, C. Boyer, J. George, T. P. Davis and J. Wang, *Small*, 2015, **11**, 2291-2304.
21. Y. Li, H. T. T. Duong, S. Laurent, A. MacMillan, R. M. Whan, L. V. Elst, R. N. Muller, J. Hu, A. Lowe, C. Boyer and T. P. Davis, *Adv. Healthcare Mater.*, 2015, **4**, 148-156.
22. F. Greco and M. J. Vicent, *Adv. Drug Delivery Rev.*, 2009, **61**, 1203-1213.
23. P. Wilson, A. Anastasaki, M. R. Owen, K. Kempe, D. M. Haddleton, S. K. Mann, A. P. R. Johnston, J. F. Quinn, M. R. Whittaker, P. J. Hogg and T. P. Davis, *J. Am. Chem. Soc.*, 2015, **137**, 4215-4222.
24. B. A. Yáñez-Martínez, J. Percino and V. M. Chapela, *J. Appl. Polym. Sci.*, 2010, **118**, 2849-2858.
25. G. Soriano-Moro, J. Percino, M. Cerón, M. E. Castro and V. M. Chapela, *J. Polym. Res.*, 2014, **21**, 492.
26. M. J. Percino, V. M. Chapela and A. Jiménez, *J. Appl. Polym. Sci.*, 2004, **94**, 1662-1669.
27. T. Zayas, M. J. Percino, J. Cardoso and V. M. Chapela, *Polymer*, 2000, **41**, 5505-5512.
28. M. J. Percino, V. M. Chapela, R. Gutiérrez-Pérez and A. M. Herrera, *Des. Monomers Polym.*, 2000, **3**, 155-160.
29. Friedheim, E. A. H.; *United States Patent*, 2422724, 1947.

