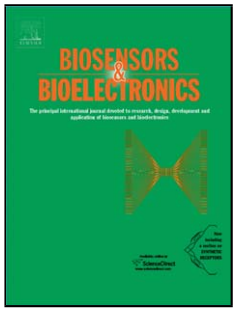
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Synthesis of controlled polymeric cross-linked coatings via iniferter polymerisation in the presence of tetraethyl thiuram disulphide chain terminator.

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Abstract

A "grafting from" approach has been used for controlled deposition of cross-linked polymers by living radical polymerisation. Borosilicate glass was modified with *N*,*N*-diethylaminodithiocarbamoylpropyl(trimethoxy)silane, in order to confine the iniferter reactive groups solely at its surface, then placed in solution with monomers and cross-linker. The polymerisation was initiated by UV irradiation. Formation of the cross-linked polymers was studied in terms of time course of the reaction, type of monomers incorporated and influence of oxygen. Grafted surfaces were characterised by AFM, FTIR, ellipsometry and contact angle measurements.

The ability to control the grafted layer improved dramatically when the chain terminator agent, *N*,*N*-*N'*,*N'*-tetraethyl thiuram disulphide (TED) was added. Upon irradiation TED increases the concentration of passive capping radicals and decreases the possibility of recombination of active macro-radicals, thus prolonging their lifetime. In the absence of TED the thickness of produced coatings was below 10 nm. TED added at different concentrations assisted in the formation of grafted layers of 10-130 nm thickness. Iniferter chemistry in the presence of TED can be used for growing nanometre-scale polymer layers on solid supports. It constitutes a robust general platform for controlled grafting and offer a general solution to address the needs of surface derivatisation in sensors technology.

Keywords

Surface initiated polymers, iniferter, surface sensor, grafting, thiuram disulphide, cross-linker.

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1. Introduction

Mastering the architecture and properties of materials at the nanometre scale is a fundamental step towards the development of leading edge tools in nanotechnology, with practical economic outcomes. Polymeric materials, in particular acrylic- and methacrylic acid based materials, have long been proven suitable for a broad range of applications: separation, recognition, sensing technologies; and are used in biomedical, environmental, security and food-related areas (Yoshida et al., 2006).

Methacrylates are reasonably well characterised and easy to manipulate. Such materials are polymerised traditionally in solution by means of initiators of radical nature through a chain reaction. Polymerisation is distinguished by: (i) free radical polymerisation, when the radical initiator is produced by homolytic cleavage of covalent bonds; (ii) ionic chain polymerisation, when cationic and anionic initiators are produced by heterolytic cleavage of ion pairs and (iii) living radical polymerisation, when radicals can be initiated or deactivated in a reversible way (Jagur-Grodzinski, 2001).

Ionic chain polymerisation is suitable for a restricted group of ionisable monomers. Free radical polymerisation is widely exploited for the preparation of commercial plastics, because it is easy to perform, leads to high yield of materials in reasonably short times, and therefore has gained a central role in polymer science. However, free radical polymerisation leads to a heterogeneous population of macromolecules, typically with a broad distribution of molecular weights and poor control over dimensions (Ward and Peppas, 2000). Among the reasons for the formation of structural heterogeneities in the growing networks, the spontaneous tendency of monomers, cross-linkers and solvent to separate during the polymerisation into uniform clusters in different spatial regions is a major factor (Kloosterboer, 1998; Anseth et al., 1996). Such segregation is defined as spinodal decomposition (Asnaghi et al., 1995), it generates local environments composed of molecules with similar properties (hydrophobicity and density) and ultimately results in different

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microgelation environments in the same polymerisation reaction and thereby gives rise to heterogeneities. On the contrary, homogeneous materials possess very desirable characteristics, e.g. optical properties, conductivity, strength and resistance etc, as evidenced by a comparative analysis of physico-chemical and functional properties of materials with dielectric spectroscopy (Kannurpatti and Bowman, 1998). The important practical need for controlled deposition of the layers of cross-linked polymers originates from the development of polymer-based sensors, especially optical sensors such as SPR or fluorescent devices. A possible way to introduce order in the polymerisation process and therefore to control it at a molecular level is to use controlled/living radical polymerisation. Living polymerisation is characterised by reversible transformation of dormant species into reactive free-radicals that act as chain propagators. In living polymerisation the rate of initiation is significantly greater than the polymerisation rate (Ruckert et al., 2002).

Among the various living iniferters (*ini*-tiator, trans-*fer* agent, *ter*-minator), dithiocarbamate esters react by the photochemical cleavage of the C-S bond (Otsu et al., 1982), leading to the formation of two radicals with very different properties. One, derived from the alkyl group, is highly reactive. It initiates polymerisation, which propagates by addition of unsaturated monomers. The other is a much less reactive (dormant), sulphur-centred radical, that does not react with monomers (Lambrinos et al., 1990; Kazmaier et al., 1995) and acts uniquely as a recapping moiety for the macro-radical growing chain. Otsu and co-workers confirmed the living nature of photoiniferter polymerisations in bulk and in solution and studied the effect of different reaction conditions on the polymerisation rate and the production of ordered polymeric chains (Otsu et al., 1995). Minimal broadening of the molecular weight distribution was observed when the chain extension process was followed by size exclusion chromatography (SEC), accounting for the formation of homogeneous macromolecules (Turner and Blevins, 1990). The kinetic behaviour of surface-initiated photo-polymerisation is quite different from that carried out in bulk or in solution and typically has a first-order dependence on monomer concentration (de Boer et al., 2000; Santosh et al., 2005). Nonlinear growth of the layers with exposure time indicates that irreversible termination

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reactions take place in the system, leading to the loss of surface-tethered free radicals. Therefore, photoiniferter-mediated surface-initiated photopolymerisation was classified as "pseudo-living" (Santosh et al., 2005; Santosh et al., 2008). An important and diverse range of materials is produced by polymerisations involving a degree of cross-linking. This results in materials with greater toughness and strength which find uses in a wide variety of applications: from dental restorative preparations (Pavlinec and Moszner, 2003) to contact lenses and optical devices (Zwiers and Dortrant, 1985), cell filtration and selective membranes (Natori and Kurita, 2007; Piletsky et al., 2000). In particular, cross-linking has practical importance in the synthesis of recognition materials based on molecularly imprinted polymers (MIPs) (Piletsky et al., 2000). In this context, iniferter chemistry has a distinct advantage, since the mild experimental conditions used in initiation by this method will help to preserve the pre-polymerisation complex, essential the for creation of recognition properties in MIPs.

Recently living iniferter photopolymerisation has been used for the development of imprinted multi-layers with predetermined selectivities (Ruckert et al., 2002; Pérez-Moral and Mayes, 2007). The present study is intended to further expand the chemistry basis of this work and provide better control over the thickness of surface-confined cross-linked layers, setting the ground for the establishment of a general platform for surface modification, which will favour the growth of surface-anchored cross-linked polymers and might benefit the polymerisation of MIPs at surfaces.

Here, we used a dithiocarbamate ester-based iniferter covalently bound to a support to grow controlled surface-grafted polymers. The 'grafting from' approach was employed: the support was first activated in order to attach the iniferter groups solely at its surface and to confine polymerisation to the surface. The ability to control the grafted layer was studied under various conditions and upon addition of a chain terminator agent, *N*,*N*-*N'*,*N'*-tetraethyl thiuram disulphide (TED), a symmetrical molecule made of two passive, dormant radicals (Otsu, 2000). The addition of TED to the monomer mixture is reported to act on the equilibrium between the active macro-radicals and passive radicals and increase the length of the surface growing chains. The overall

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architecture strategy is summarised in Scheme 1. We investigated the effect of TED on polymer growth, in order to control grafting thickness over the range 5-130 nanometres. The aim was to find a recipe for the growth of nano-scale polymer layers of controlled thickness. The ability to predictably grow such layers would help in the design of recognition layers compatible with sensor systems. Moreover it would aid in the design of multi–layer architectures, consequently allowing the optimisation of electrical conductivity and improved diffusion of analytes thus enabling the preparation of a new generation of highly sensitive polymer-based sensors.

2. Experimental

2.1. Materials

Sodium diethyl dithiocarbamate trihydrate, chloropropyl trimethoxysilane, acetone and 9-anthracenylmethyl methacrylate were purchased from Sigma-Aldrich (Gillingham, UK). All other chemicals were of analytical grade and were used as received. Ultrapure water (Millipore) was used for analysis. NMR spectra were obtained using a JEOL ECX-400 multinuclear NMR spectrometer. NMR solvents were obtained from Goss Scientific (Chelmsford, UK).

2.2 Synthesis of *N*,*N*-diethylaminodithiocarbamoylpropyl(trimethoxy)silane

The synthesis of the silane living iniferter, *N*,*N*-diethylaminodithiocarbamoylpropyl(trimethoxy)silane was prepared by a modification of the procedure of Kobayashi et al (1992). Diethyl dithiocarbamate trihydrate (3.42 g) was ground to a fine powder in a mortar. The powdered salt was transferred to a flask and the water of crystallisation removed by heating in an oil bath (105°C), while connected to a vacuum pump for 10 hours. Dehydrated dithiocarbamate salt (2.65 g) was suspended in 40 mL of dry acetone in a flask, fitted with a stirrer and condenser, to which was added an amount of chloropropyl trimethoxysilane (2.48 g) to give a 1.2:1 mol:mol ratio of iniferter:silane. The mixture was heated at 45-55°C. Product, in the form of a yellow oil, was formed after 10-15 hours. At the end of the

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reaction, the insoluble salts were removed by filtration, and the solvent evaporated at 40 °C under vacuum. Product formation and purity were confirmed by TLC and NMR. A quantity of 3.89 g of product was formed.

2.3. Activation of borosilicate supports

Support surfaces were borosilicate glass slides cut into rectangles of 0.7 x 0.7 cm. Surface activation was achieved by the following steps: 10 min ultrasonication in water, 10 min ultrasonication in 1 M NaOH, 10 min in water. Then, 10 min ultrasonication in 5 M HCl, 10 min ultrasonication in water and 10 min ultrasonication in acetone. Finally surfaces were dried for 2 hours in an oven at 80°C.

2.4. Derivatisation of borosilicate supports with silane living iniferter

Activated borosilicate supports were functionalised by immersion for different times (from 1 to 12 hours) in a 2% v/v solution of *N*,*N*-diethylaminodithiocarbamoylpropyl(trimethoxy)silane in toluene. The supports were then washed in methanol and dried at room temperature. Derivatisation was confirmed by static contact angle measurements.

2.5. Analysis of silane modification by contact angle

Static contact angle (CA) measurements were performed on a Cam 100 optical Angle Meter (KSV Instruments Ltd., Finland). Measurements were repeated 4 times and the mean value calculated.

2.6. Quantitative estimation of the level of silane modification on the supports

Iniferter-modified borosilicate slides and control slides (not derivatised with the iniferter) were placed in a glass Petri dish filled with 20 ml of a 1% fluorescent monomer, 9-anthracenylmethyl acrylate in toluene, ultrasonicated for 10 min and purged with argon for 20 min. UV irradiation (Philipps UV lamp 9 W/cm^2) was carried out for 20 and 40 min. After the polymerisation, slides

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were washed in methanol and dried.

2.7. Polymer grafting

For the grafting, the iniferter-modified supports were placed in a covered Petri-dish and immersed in the polymerisation mixtures: (P3) 0.5g MAA, 4.5g EGDMA, 5g toluene; (P4) 0.46g 2-TFMAA, 4.29g EGDMA, 5g toluene; (P5) 0.665g EGMP, 4.08g EGDMA, 5g toluene. After removal of oxygen by purging with argon, the polymerisation was carried out for different times (0, 10, 20, 40, 60, 80 min) using a Phillips UV lamp (9 W/cm^2), mounted at 8 cm distance from the surface. Polymer grafting was also performed with the addition of tetraethylthiuram disulphide (TED) at the concentrations 0, 100 nM, 1 μM, 10 μM and 100 μM. Experiments were repeated in a modified atmosphere, inside an Atmosbag continuously purged with nitrogen. The grafted supports were washed with methanol, dried and analysed with contact angle (CA), AFM and FT-IR.

2.8. Fluorescent analysis of the grafted layer

The fluorescent monomer 9-anthracenylmethyl acrylate in the concentrations 0, 0.031, 0.0625, 0.125, 0.25, 0.5 and 1% was added to a solution containing 0.1 g MAA, 0.9 g EGDMA, in 1 g toluene, sonicated for 10 min and then purged with argon for 20 min. The slides with the initiator attached to the surface were placed in covered Petri-dishes and immersed in the polymerisation mixtures. The covalent attachment occurred under UV light during 20 min. The surfaces were then washed with methanol and dried under a stream of nitrogen.

Quantification of the fluorescence of the surface was performed with a fluorescence analyser equipped with GeneSnap and GeneTool softwares (Syngene Europe, Cambridge, UK). Images were captured at fixed readout wavelength (λ_{exc} 320nm, λ_{em} 410nm), 0.200s exposure, 20 RU radius. A calibration curve was plotted with the relative fluorescence units (RFU) and the quantity of monomer added to the mixtures. The equation for the calibration was linear, y (RFU) = 5.7865 x (μg of fluorescent monomer). The fluorescence readouts were converted into μg/ area and into

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mol/ area.

2.9. FT-IR analysis of the grafting & AFM analysis

FT-IR spectroscopy was used for the qualitative analysis of the grafted polymers. The equipment used was a Bruker IR-Scope-II (Bruker Optics, UK) equipped with attenuated total reflection (ATR) objective and controlled by Opus/IR optic software. FT-IR spectra were collected in reflectance mode, in the range between 600 and 4000 cm^{-1}, subtracted for the background. The different preparation steps were analysed. AFM images were obtained in contact mode using the PicoScan™ instrument (Molecular Imaging Corporation, Tempe, AZ, USA). Surface topography was analysed by scanning regions of 10 x 10 μm^2 and 1 x 1 μm^2.

2.10. Ellipsometry measurements

A Beaglehole Instruments phase-modulated Picometer ellipsometer (He−Ne laser, λ = 632.8 nm) was used to measure the thickness of dry layers of the grafted polymers.

A refractive index of 1.48 was used for polymer layers. The ellipsometric angles Ψ and Δ were measured at angles of incidence ranging from 80° to 35° (measurements were made every 1.00 degree), and fit using a Cauchy model (Igor Pro. software package) to obtain the thickness with an accuracy of ± 1 nm. Thickness measurements were taken at five different points on every sample measured in ambient air.

3. Results and Discussion

3.1. Derivatisation of the supports with initerfer

The silane iniferter *N*,*N*-diethylaminodithiocarbamoylpropyl(trimethoxy)silane was synthesised with a modified protocol adapted from Kobayashi *et al*. (1992) (Supplementary Material 1). Advantages of the modified protocol are the ease of purification and high yield (76%) of product.

Support surfaces were modified with the silane-living iniferter after activation. The time course of

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the modification was studied and optimised by static CA measurements. Bare silica has a CA of 10-12° that changed to ca. 80° due to the modification. The changes in CA measured were plotted as percentage versus time, as reported in Figure 1. Results displayed a saturation course and were in line with the literature (Kobayashi et al. 1992). A period of 3 hours was considered satisfactory for the derivatisation process.

An estimation of the quantity of iniferter groups placed on the supporting surface was attempted by using fluorescence, in line with other quantitative fluorescence surface studies (Ivanov et al., 1996; Xing at al., 2007). It was supposed that the activation of the iniferter groups in the presence of fluorescent monomers would result in labelling the majority of the iniferters, thus offering the possibility to estimate the quantity of active iniferters on the surface. The photo-activation was allowed to proceed for different times (20 and 40 min) on supports derivatised with silane iniferter and on controls. The results are summarised in Table 1 with batches showing variability in the range 10-15%, possibly due to self aggregation of the fluorescent monomer as a source of error. Data for 20 or 40 min photo-activation did not vary significantly, probably indicating that the grafting of the layer of monomers onto the surface is a fast process that occurs within the first few minutes. Longer irradiation times resulted greater variability in the results, presumably due to other phenomena, such as self-polymerisation of the monomers in solution, or loss of iniferter groups at the surface occurring, thus confusing the experimental picture. Upon ultrasonication the quantity of active iniferter groups attached to the surface dropped by 50% with respect to non-ultrasonicated supports, suggesting that loosely attached monomers and iniferter are removed in the process. The quantity of grafted iniferter groups was estimated as 20-30 pmol/ mm^2.

3.2. Polymer grafting

A layer of polymer was grown from the surface. The activation of the polymerisation process was by UV photodecomposition of the iniferter attached to the support. Ellipsometry measurements indicated that 20 min UV irradiation gave rise to ~ 5 nm polymer thickness, 40 min UV gave rise

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to ~ 6 nm polymer thickness. Time seemed scarcely relevant for determining the thickness of the grafted layer and no variations were detected between the different monomer compositions tested. CA measurements were reported in Figure 2 A, showing an angle of about 68° for the grafted polymer, which is in accordance with the literature data (Yuan et al., 2007).

In order to characterise the grafted layers, FT-IR spectra of the supports after each derivatisation step were collected. Figure 2 B shows the spectra of the iniferter-modified support and of the grafted polymers P3, P4 and P5. Iniferter-modified supports displayed a characteristic peak at 1650 cm^{-1} for the CS group and some peaks in the region around 1200 cm^{-1}, attributed to thiocarbamyl (C=S) stretching (Fig. 2 B, a). As expected, the contribution of the dithiocarbamate iniferter, including the presence of CH_3 and CH_2 vibration is seen between 1100 and 1550 cm^{-1} (Han and Williams, 1991).

In the case of polymers P3, P4 and P5, the wavenumber of the C=O stretching vibrations in the region of 1900–1200 cm^{-1} is a function of the copolymer composition (Ruckert et al., 2002). The spectra showed the C=O stretching vibration, typical of the ester/acid carbonyl groups, at ~1732/1710 cm^{-1} (Fig. 2 B, arrows, b). The asymmetric carboxylate stretching band $\nu_{as}(COO^{-})$ is observed near 1560 cm^{-1} (Fig. 2 B, c) (Colthup et al., 1975).

Polymer P3 contained methacrylic acid with CH_3 and CH_2 groups giving stretching absorptions at 2998 and 2954 cm^{-1} respectively. The deformation bands of these groups were observed at 1487 and 1452 cm^{-1}. The C=O stretching around 1700 cm^{-1} was also observed. Polymer P4 contained the trifluoromethyl acrylic monomer distinguished by the presence of intense signals at 1250 cm^{-1} (Fig. 2 B, d). Polymer P5 contained the phosphate monomer, which gave an intense signal in the region 1250-1350 cm^{-1} (Fig. 2 B, f). These results prove the different natures of the grafted polymers.

3.3. Growing grafted polymers with controlled thickness

The goal of this work was to achieve control over the layer of cross-linked polymer grafted to the

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surface. Producing a grafted polymer of a defined thickness has practical importance, e.g. in assuring the formation of an adequate number of binding sites, as in the case of polymer used in sensor development.

There are different factors responsible for polymer growth and therefore important for controlling the process and extending the grafted layer of significant dimensions: (i) the amount of iniferter on the surface (Lin, 2001), (ii) the reactivity of the monomer and the cross-linker (Ward et al., 2002), (iii) the stability of the radicals (Ward et al., 2002), (iv) the decrease of the polymerisation rate at the surface due to diffusional and steric barriers (Blaya et al., 2004), (v) the presence and the incorporation of oxygen in the solution during the polymerisation, (vi) the equilibrium between active/dormant species (Mijangos et al., 2006).

We took into consideration the presence of oxygen and the equilibrium between active/dormant radical species. Figure 4 indicated that an oxygen-free environment is essential for the growth of the polymer. The thickness of P3 obtained from solutions simply purged with argon was 6 nm, while polymerisation in better-controlled atmosphere within an Atmosbag produced a coating of 20 nm thickness.

In the second case, the equilibrium between active/dormant radicals was shifted by the addition of a chain terminating agent, tetraethyl thiuram disulphide (TED) (Otsu, 2000). The terminating agent TED was added to the monomer mixture at the concentration of 10 μM and polymerisation was carried out for 80 min. Ellipsometry was used to determine the thickness of the grafted polymer layer. Results shown in Figure 3 A indicated that P3 reached the significant thickness of ca. 100 nm. It could be hypothesised that 10 μM of TED in the monomer solution would prevent the irreversible recombination of active radicals by providing an excess of dormant radicals, thus prolonging the life-time of the surface grafted macroiniferter.

The influence of the polymerisation time on polymer thickness was evaluated. Figure 3 B shows that increased irradiation time corresponds with an increased thickness of the grafted polymer layer. It was noted that 40 min polymerisation produced a thickness of about 70 nm, while 80 min

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produced a 100 nm layer. The relationship between irradiation time and thickness of the grafted layer was almost linear (equation: y(nm)=1.3857x(min of polymerisation); R^2=0.8952), this observation is in accordance with other surface macromolecular architectures prepared by photochemical initiation (Nakayama and Matsuda, 1996) and accounts for a limited disruption of the surface proximity to the chain elongation process. Further investigations were conducted to determine whether there was a correlation between the concentration of TED and the thickness of the grafted polymer. Figure 4 shows the dependency of the grafted polymer thickness on the concentration of the chain terminating agent, TED. The irradiation time was fixed at 40 min. A direct correlation between the concentration of TED and thickness of polymer emerged, indicating that manipulation of the quantity of TED in solution is an effective way to obtain grafted layers with thicknesses between 5 and 130 nm. A plateau effect seemed to occur for high concentrations of TED. The data were supported by FT-IR and AFM studies.

4. Conclusions

In conclusion, the versatility of living initiators to prepare grafted layers was recently demonstrated in the work on functionalised nanoparticles, including molecularly imprinted polymers (Ruckert et al., 2002). Subsequently, the possibility to polymerise consecutively different layers, each one incorporating different properties as desired was also demonstrated (Pérez-Moral and Mayes, 2007). Control of polymerisation allows for the design of customised multiple-functionality layers, one on the top of the other and offers practical solutions for biosensors and biomimetic applications on chips.

In order to understand and have control of the growth of cross-linked polymeric surfaces, our research covered the basic aspects of the production of polymer surfaces using living iniferter mediated polymerisation. Monitoring each preparation step and the polymerisation with FT-IR allowed a close control of the grafting process as the distinctive vibrational signals for iniferter and for polymeric materials were identified. The grafting was directly dependent on the irradiation

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time and on the concentration of chain terminating agent. The best conditions for grafting so far identified were 40-60 minutes of irradiation and 10 to 100 μM of TED, which allowed the growth of a 100 nm thick polymer layer, while a 100nM TED concentration resulted in the formation of 10 nm-thick polymer film. The process could in principle be extended to any composition of polymer desired, including MIPs. Such an approach would help to design ideal or highly compatible recognition layers to be integrated within sensor systems, optimising electrical conductivity and reducing diffusion times for the analytes, thus enabling the preparation of a new generation of highly sensitive polymer-based sensors. In conclusion we have demonstrated an ability to enhance control over the thicknesses of polymeric coating made of cross-linked materials.

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Appendix A. Supplementary materials

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Figure Captions

Figure 1. Time course of the derivatisation of borosilicate supports with silane living iniferter.

Figure 2. A) Contact angle measurements as evidence for the grafting of the different polymeric compositions. B) FT-IR spectra of the different polymers.

Figure 3. A) Thickness of the grafted polymer layer. B) Time dependence of the thickness of the grafted layer. Solid bars indicate P3 + TED 10 μM. White bars indicate control experiment in the absence of TED.

Figure 4. Relationship between the concentration of TED in solution and the thickness of the grafted layer.

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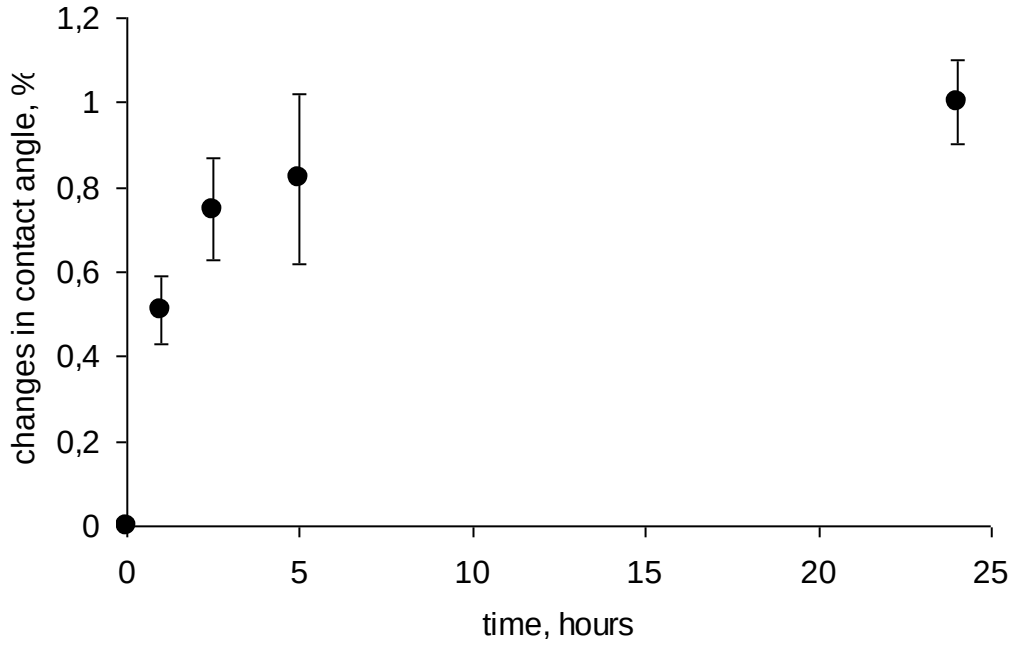
Table 1. Quantity of active iniferter on the surface.

Sample description	UV exposure	Ultrasonication after polymerisation	Fluorescent monomer, μg/mm^2	Fluorescent monomer, pmol/mm^2
Control	20 min	No	0.012 ± 0.002	40 ± 7
Iniferter	20 min	No	0.021 ± 0.003	74 ± 11
Control	40 min	No	0.011 ± 0.001	45 ± 4
Iniferter	40 min	No	0.033 ± 0.002	87 ± 5
Control	20 min	5 min	0.005 ± 0.001	19 ± 4
Iniferter	20 min	5 min	0.007 ± 0.001	28 ± 4

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Figure 1. Bossi et al.

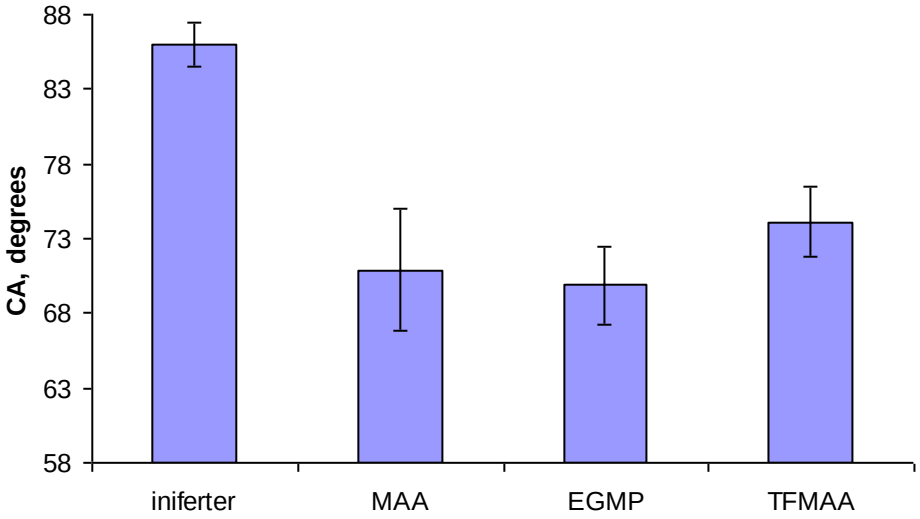


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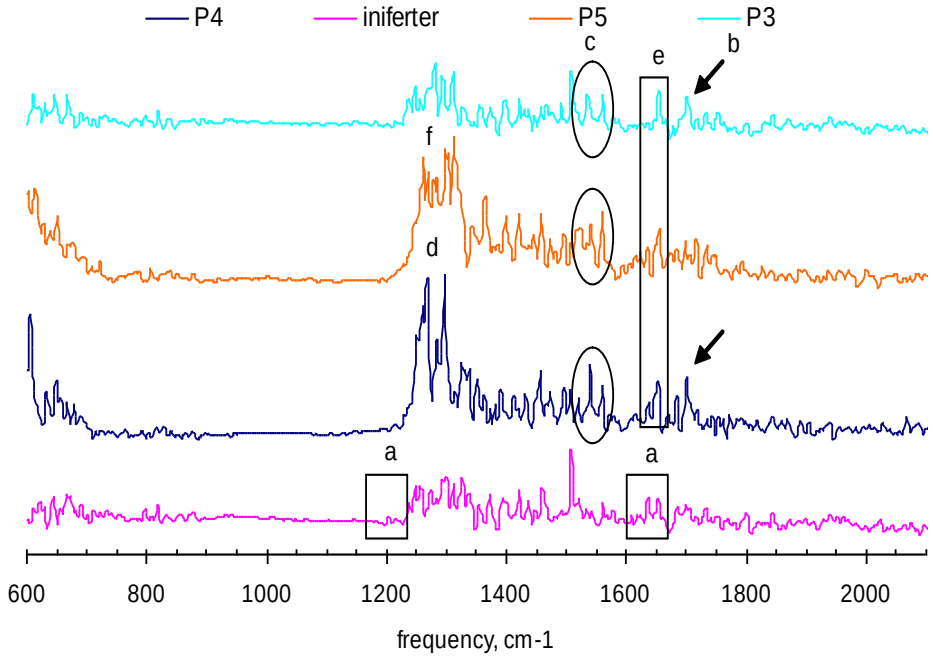
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Figure 2. Bossi et al.

A



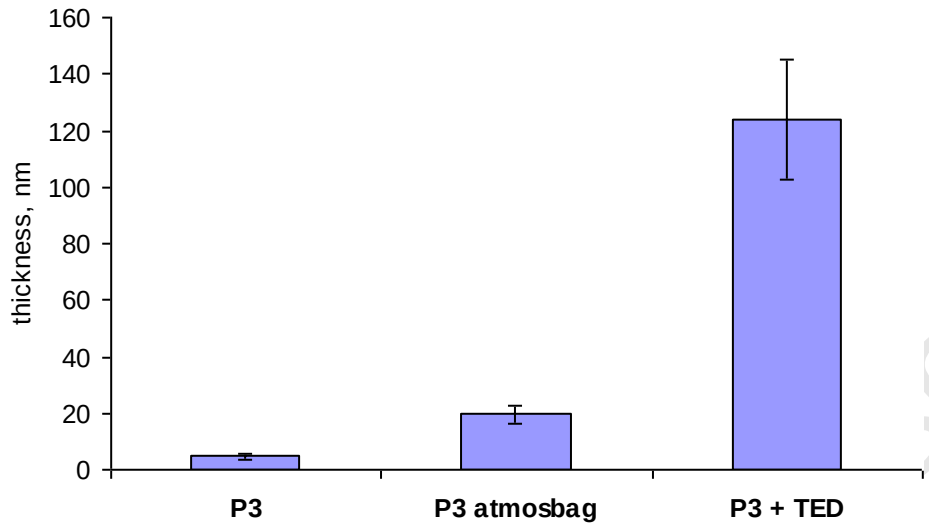
B



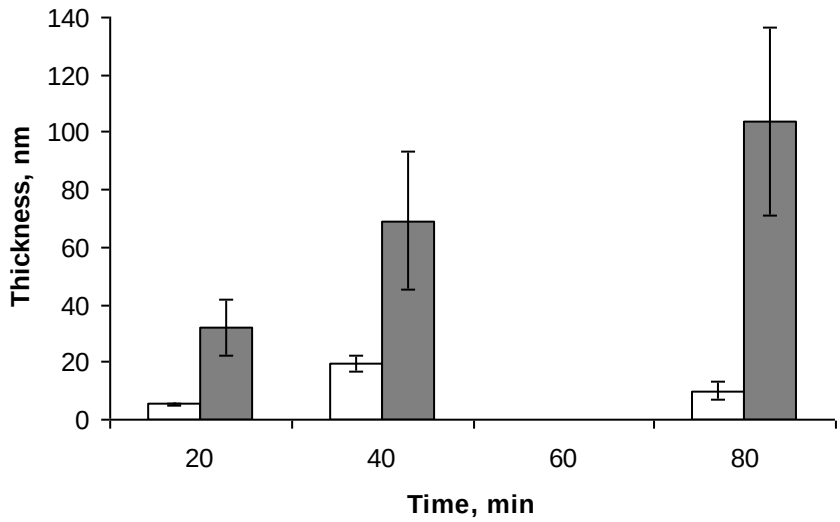
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Figure 3. Bossi et al.

A.

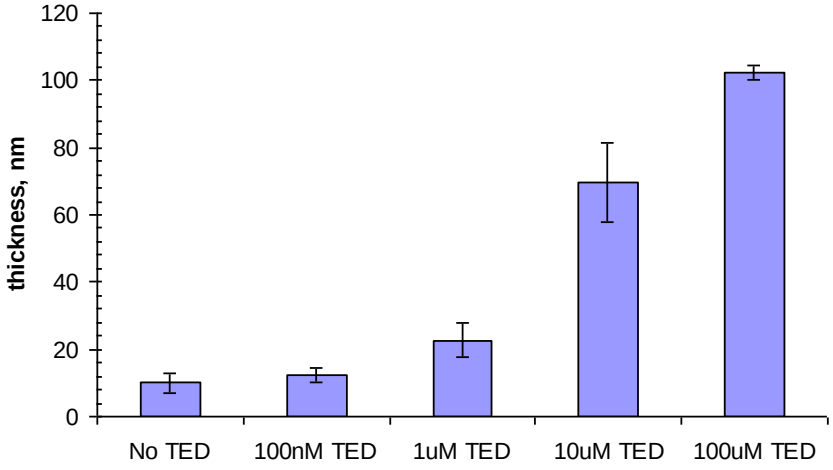


B.



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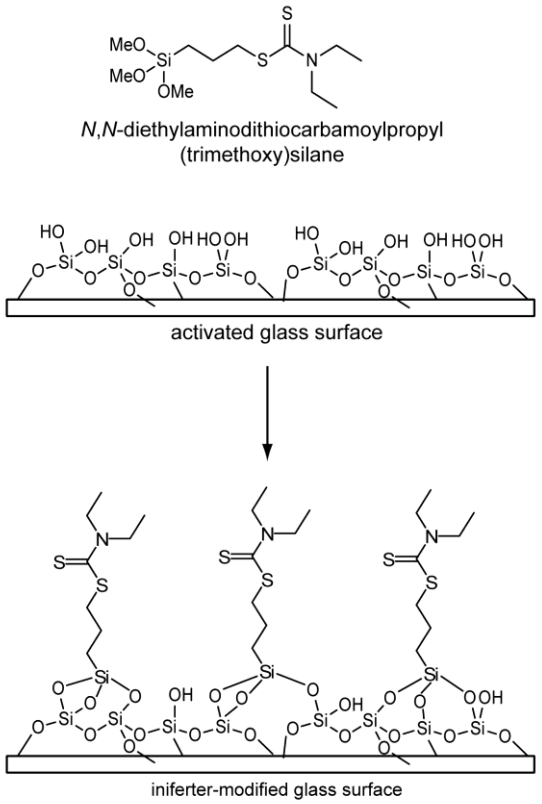
Figure 4 Bossi et al.



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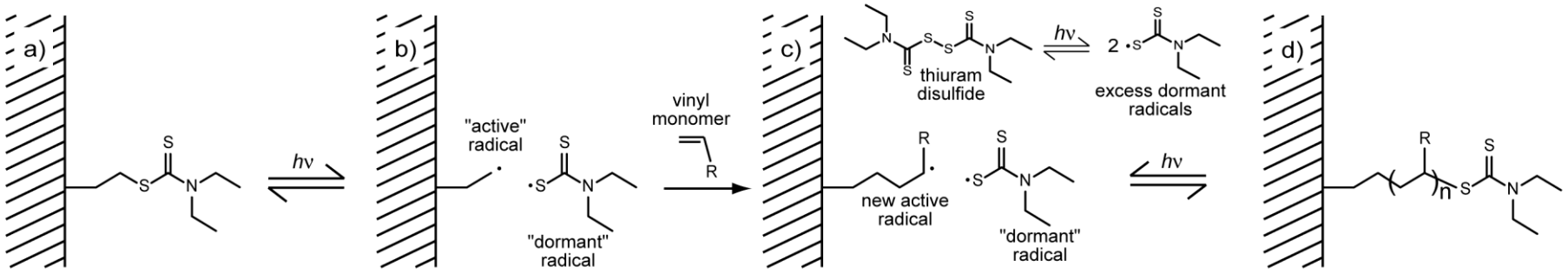
Scheme 1. A) Iniferter immobilisation.



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Scheme 1. B) Polymerisation at surface.



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Synthesis of controlled polymeric cross-linked coatings via iniferter polymerisation in the presence of tetraethyl thiuram disulphide chain terminator

Bossi, Alessandra; Whitcombe, Michael J.; Uludag, Yildiz; Fowler, S.; Chianella, Iva ...

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