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ARTICLE

Experimental model design: Exploration and optimization of customized polymerization conditions for the preparation of targeted smart materials by click Diels Alder

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The experimental model design proposed herein has proved to be an indispensable tool to rapidly and easily elucidate the optimal polymerization conditions in the preparation of tailor-made responsive materials for biomedical applications. The use of reversible covalent bonds for the synthesis of new materials provides the polymer structures with novel functionalities and applications. Diels-Alder (DA) reaction is an ideal synthetic method for the preparation of functional and responsive biomaterials under mild and undemanding conditions. The present work details the optimization of the polymerization conditions to synthesize two families of thermo-sensitive polymers, either with reduction-responsive properties, or with enhanced hydrophilicity. These polymers have been successfully prepared from two functional bisdiene monomers: a difurfuryl dithiodiethanol derivative (DiT-Fur, **1**) and a difurfuryl-dithiothreitol derivative (DTT-Fur, **2**), respectively, using the same bismaleimide (DMDOO, **3**) derived from triethylene glycol in both systems. Twenty experiments were conducted and a Box–Behnken experimental design used to evaluate the importance of temperature and water content in terms of the polymer molecular weights (M_n , determined by ¹H NMR). In addition, the progress of the polymerizations at selected times was accurately monitored via IR spectroscopy. Higher temperatures were discarded since previous thermo-lability studies demonstrated that the retro-DA reaction could occur at temperatures as low as 50 °C. Temperature proved to be the most influential variable in final M_n , exerting opposite influences in both systems. We envision that this experimental approach, applied to other polymerization processes, can enlighten the influence of key parameters in the final product obtained.

Introduction

Over the last few years, increasing attention has been dedicated to the use of new polymerization techniques that meet the three main criteria of an ideal synthesis: efficiency, versatility, and selectivity.¹ There is also a need for the construction of functional and responsive tailor-made materials² for their application in a wide range of fields, especially in biomedicine.³ The use of reversible covalent bonds in the preparation of polymeric materials is an attractive tool that allows changes in their arrangement and bond structure sparked off by an external stimulus such as heating, light or pH, while retaining their stability in the absence of trigger. In recent years, more and more research has been devoted to the utilization of reversible covalent bonds in synthesizing new materials,⁴ which not only overcomes disadvantages of permanent covalent polymers, but also brings in new functionalities such as self-

healing, smart drug delivery and controllable degradation, broadening vastly the scope of use of these materials.⁴

Among the group of reactions capable of forming reversible covalent linkages, Diels-Alder (DA) has emerged as a click reaction that provides an immense number of opportunities and advantages, making it an ideal candidate for its use in the preparation of functional and responsive biomaterials. Click Chemistry (CC) has emerged as a wide-spread approach that uses only the most-practical and -reliable chemical transformations, with an explosive growth in publications in recent years.^{5–8} The term CC was coined by Sharpless and col.⁵ According to the authors, a reaction should meet a set of stringent criteria to be defined as a click reaction. Thus, “*the reaction must be modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by nonchromatographic methods, and be stereospecific (but not necessarily enantio-selective). The required process characteristics include simple reaction conditions (ideally, the process should be insensitive to oxygen and water), readily available starting materials and reagents, the use of no solvent or a solvent that is benign (such as water) or easily removed, and simple product isolation. Purification —if required— must be by nonchromatographic methods, such as crystallization or distillation, and the product must be stable under physiological conditions*”.⁵ Although fitting the requirements of a click reaction is a tall order, several processes did so. Thus, the DA-

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coupling reaction is a well-established click reaction that combines a diene moiety, such as furan rings and a dienophile group, such as maleimide rings, in a robust, efficient, and orthogonal method for the functionalization of different compounds.^{1,9} The chemoselectivity of DA reactions is narrowly defined, that is, it is orthogonal to an unusually broad range of reagents, solvents, and other functional groups such as the –COOH, –OH and –NH₂ groups found in biomolecules. It is also moisture and oxygen tolerant and, under mild conditions, does not generate side products. As the click reaction that it is, the yields are usually very high, and the reaction can be carried out under particularly mild conditions (aqueous solution, neutral pHs and moderate temperatures). Moreover, that DA reactions can proceed in the absence of metal catalysts is an extra bonus.¹⁰ Consequently, this reaction allows clean, reliable and sequential transformations of wide scope.⁵

DA cycloaddition reactions have demonstrated to provide benefits in a variety of fields and systems such as in 3D laser micro- and nanoprinting,¹¹ in bioconjugation procedures, immobilization of oligonucleotides, protein, peptides, carbohydrates and antibodies,^{1,12} in the preparation of smart drug delivery systems (DDS),¹³ in the core-stabilization of nanoparticles to prevent their disruption by dilution in the organism,^{14,15} in the gelation processes of hydrophilic polymers,¹⁶ and in the preparation of brush polymers¹⁰, self-healing materials^{17,18} and functional polymers¹⁹ among others. One of the preferred DA systems is the reaction between furan and maleimide moieties. This combination has been widely used since its DA adducts are formed at moderate temperatures and can be reverted back to the starting maleimide and furan pair at elevated temperatures.²⁰ A wide variety of reaction conditions have been found in the scientific literature among furan and maleimide derivatives. Thus, for example, it has been carried out in many solvents: from toluene,¹⁰ water and chloroform,^{16,19} and tetrahydrofuran²¹ up to in bulk.¹ Depending on the conditions chosen, coupling reactions proceed from a few hours to days, at temperatures as varied as 30 °C, 40 °C, 60 °C or even at 120 °C in a retro-DA procedure.²¹ Monomer concentration usually ranges from 7.0 to 9.0 mM^{10,19} and the reaction can be speeded up by the use of a number of catalysts.^{22–24}

Taking into account the findings from published scientific literature,^{9,20} temperature seems to be a key parameter among the reaction conditions since, together with the acceleration of the DA reaction, the retro-DA reaction can occur simultaneously, affecting the latter to the overall results. Additionally, water has demonstrated to exert a unique effect in this type of reactions, not only in its kinetics but also in its stereoselectivity²⁵ as was demonstrated in the early 1980s by the research teams headed by Breslow²⁶ and Grieco.²⁷ These findings can be considered the “Big Bang” in aqueous synthesis and triggered the general interest in the use of water as a solvent in organic chemistry.²⁵ Surprisingly, and as far as the authors are aware, there is no systematic study on the influence of both independent variables (temperature and water content) on DA polymerizations of furan-maleimide monomers and their impact in the final materials.

The main goals of the present work are, on the one hand, to find the optimal conditions for the preparation of functional and responsive polymers with potential use in biomedicine through a Box–Behnken experimental design; on the other hand, to determine the relative influence of the chosen variables (temperature and water content) and to verify whether the general trends observed for one system can be generalized or, on the contrary, depends on the system studied. Additionally, the scope and limitations of the use of IR spectroscopy, via the second derivative of the absorption spectra, will be investigated as a tool to track the degree of conversion of the monomers in the polymer syntheses.

Experimental

General Methods.

All chemicals used were purchased from Aldrich Chemical Co. Thin layer chromatography (TLC) were performed on silica gel 60 F₂₅₄ (Merck), with detection by UV light charring with *p*-anisaldehyde, phosphomolybdic acid or ninhydrin. Silica gel 60 (Merck, 40–60 and 63–200 μm) was used for preparative chromatography. Melting points were determined in a Bibby Steriling LTD device, in open capillary tubes and are uncorrected. Gel permeation chromatography (GPC) analyses were performed using a Waters apparatus equipped with a Waters 2414 refractive index detector and two Styragel® HR columns (7.8 x 300 mm²) linked in series, thermostatted at 40 °C, and using *N,N*-dimethylformamide (DMF) containing LiBr 5.8 mM as the mobile phase at a flow rate of 1.0 mL/min. Molecular weights were estimated against polystyrene standards. The thermal behavior of the polymers was examined by Differential Scanning Calorimetry (DSC), using a TA DSC Q-200 Instrument calibrated with indium. DSC data were obtained from samples of 1–5 mg at heating/cooling rates of 10 °C min⁻¹ under a nitrogen flow (flow rate 50 mL min⁻¹). The glass transition temperatures were determined at a heating rate of 10 °C min⁻¹ from rapidly quenched polymer samples. Thermogravimetric analyses (TGAs) were performed under a nitrogen atmosphere (flow rate 100 mL min⁻¹) with a Universal V4.3A TA Instrument at a heating rate of 10 °C min⁻¹. For stability studies of the Diels–Alder adduct bonds, isotherms at 50 °C, 60 °C and 80 °C were conducted. In addition, ramps at several heating rates were carried out for the model compound **4**. The NMR and mass spectra were registered in CITIUS (University of Seville). ¹H and ¹³C NMR spectra were recorded at 300 K with a Bruker AMX-500 and a Bruker Advance AV-500 for solutions in CDCl₃ and DMSO-*d*₆. Chemical shifts (δ) are reported as parts per million downfield from Me₄Si and *J* in Hz. *J* are assigned and not repeated. All the assignments were confirmed by COSY and HSQC experiments. High resolution mass spectra were recorded on a Q-Exactive spectrometer.

Preparation of monomers and small molecules. Experimental procedures.

Disulfanediyl bis(ethane-2,1-diyl) bis[(furan-2-ylmethyl)carbamate]. Furfuryl derivative of 2,2'-Dithiodiethanol (DiT-Fur (1)). A round bottom flask loaded with 2-hydroxyethyl disulfide (DiT, 616 mg, 4 mmol) was subjected to three cycles of vacuum-argon and dry THF (2 mL) was added to get a solution. Then, furfuryl isocyanate (0.85 mL, 984 mg, 8 mmol) was added followed by one drop of the catalyst dibutyltin dilaurate. After stirring 1.5 hours at room temperature, a solid was formed. The stirring was kept for further 5 hours and then the reaction mixture was concentrated to dryness. The residue was purified by column chromatography using ethyl acetate-hexane 1:3 as eluent and the title compound was obtained as a white solid (672 mg, 42%). M.p. 96-98 °C.

FTIR-ATR: ν (cm⁻¹) 3307 N-H; 3060 =C-H; 1681 C=O; 1540 δ N-H; 1505 C=C; 1256 δ C-N.

¹H NMR (DMSO-*d*₆, 500 MHz): δ (ppm) 7.68 (t, 2H, H_{NH}, J_{NH,e} = 5.5 Hz), 7.54 (d, 2H, H_a, J_{a,b} = 2.0 Hz), 6.37 (dd, 2H, H_b, J_{b,c} = 3.0 Hz), 6.21 (d, 2H, H_c), 4.20 (t, 4H, H_f, J = 6.5 Hz), 4.16 (d, 4H, H_e), 2.95 (t, 4H, H_g). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ (ppm) 155.7 (C=O), 152.2 (C_d), 141.5 (C_a), 110.2 (C_b), 106.3 (C_c), 61.6 (C_f), 37.3 37.1 (C_{e,g}) (see Supplementary Information, ESI).

ESI-Mass Spectrometry: *m/z* 423.06 [M + Na]⁺. HR-ESI-MS *m/z*: calculated from C₁₆H₂₀O₆N₂S₂Na = 423.0655; found = 423.0648 (difference between the theoretical and experimental values: 1.6498 ppm).

S,S'-(2,3-dihydroxybutane-1,4-diyl) bis[(furan-2-ylmethyl)carbamothioate]. Furfuryl derivative of D,L-Dithiothreitol (DTT-Fur (2)). To a solution of dithiothreitol (DTT, 308 mg, 2 mmol) in THF (7 mL), triethylamine (0.14 mL, 1 mmol) was added and stirred for 5 min. The solution was cooled at 0 °C and then, a second solution of furfuryl isocyanate (0.58 mL, 5.44 mmol) in THF (6 mL) was added dropwise. The reaction mixture was allowed to warm up slowly to rt and proceeded for 6 hours. The solvent was removed under vacuum and the residue dissolved in ethyl acetate (100 mL). The organic layer was washed with an aqueous solution of sulfuric acid (0.3 M, 2 x 20 mL), dried with sodium sulfate and the solvent evaporated under reduced pressure leading to a colored solid. The solid was washed with warm dichloromethane leading to the title compound as a pure white solid (358 mg, 45%). M.p.: 129 °C-131 °C.

FTIR-ATR: ν (cm⁻¹) 3265 N-H, O-H; 3018 =C-H; 1631 C=O; 1520 δ N-H; 1505 C=C; 1211 δ C-N.

¹H NMR (DMSO-*d*₆, 500 MHz): δ (ppm) 8.60 (bs, 2H, NH), 7.58 (bs, 2H, H_a), 6.40 (bs, 2H, H_b), 6.25 (bs, 2H, H_c), 4.85 (d, 2H, OH, J_{OH,g} = 6.0 Hz), 4.30 (d, 4H, H_e, J_{e,NH} = 3.0 Hz), 3.49 (bd, 2H, H_f), 2.98 (dd, 2H, H_g, J_{g',g''} = 13.5 Hz; J_{g',f} = 5.0 Hz), 2.89 (dd, 2H, H_{g''}, J_{g'',f} = 7.5 Hz). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ (ppm) 166.2 (C=O), 151.8 (C_d), 142.2 (C_a), 110.4 (C_b), 107.1 (C_c), 71.6 (C_f), 37.1 (C_e), 32.3 (C_g) (see ESI).

ESI-Mass Spectrometry: *m/z* 423.06 [M + Na]⁺. HR-ESI-MS *m/z*: calculated from C₁₆H₂₀O₆N₂S₂Na = 423.0655; found = 423.0651

(difference between the theoretical and experimental values: 0.8347 ppm).

2,2'-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione) (DMDOO-Furan (4)). A mixture of 1,8-dimaleimide-3,6-dioxaoctane (DMDOO (3), 125 mg, 0.41 mmol) and furan (0.12 mL, 1.62 mmol) was dissolved in dichloromethane (1.25 mL) and was stirred at 20 °C for 7 days. The solvent and the excess of furan were removed under vacuum and the title compound was isolated as an uncolored oil in quantitative yields.

FTIR-ATR: ν (cm⁻¹) 3006 =C-H; 1690 C=O.

¹H NMR (CDCl₃, 500 MHz) δ (ppm) 6.50 (bs, 4H, H_b *exo*), 6.39 (bs, 4H, H_b *endo*), 5.31 (bs, 4H, H_a *endo*), 5.25 (bs, 4H, H_a *exo*), 3.70-3.40 (m, 28H, H_h, H_i *endo*), 2.85 (bs, 4H, H_i *exo*). Ratio *endo/exo*: 43:57. ¹³C NMR (CDCl₃, 125 MHz): δ (ppm) 176.1, 174.8, (C=O amide), 136.5 (C_b *exo*), 134.4 (C_b *endo*), 80.9, (C_a *exo*), 79.5 (C_a *endo*), 70.1, 69.9, 67.1, 67.0, 38.3, 37.7 (C_h), 47.5 (C_i *exo*), 46.0 (C_i *endo*) (see ESI).

HR-ESI-MS *m/z*: calculated from C₂₂H₂₄O₈N₂Na = 467.1425; found = 467.1417 (difference between the theoretical and experimental values: -1.6787 ppm).

Thermal stability studies of the model DA adduct DMDOO-Furan (4). Kinetic parameters determination under model-based analyses.

A model-based analysis was used to find the activation energy of the thermally induced retro-Diels-Alder reaction of compound 4 under the acceptance of a kinetic model.²⁸ Three assumptions have been made for the reaction under study. Firstly, it was assumed that reactions are the result of several elementary reaction steps; the reaction rate of each step can be described by its own kinetic equation $f(\alpha)$, which in turn depends on mass conversion (α). The kinetic equation of each elementary step $f(\alpha)$ is linked to the initial concentration of the reactant and the concentration of the product [Equation (1)]:

$$\frac{d\alpha}{dt} = k f(\alpha) \quad (\text{Eq. 1})$$

The rate constant "k" obeys to the Arrhenius law [Equation (2)]:

$$k = A e^{-\frac{E_a}{RT}} \quad (\text{Eq. 2})$$

where "k" is the rate constant, "T" is the temperature (in kelvins), "A" is the pre-exponential factor—a constant for each chemical reaction—, "E_a" is the activation energy for the reaction, and "R" is the universal gas constant.

Secondly, it was presumed that the kinetic parameters kept constant during the reaction progress for each individual reaction step and thirdly, it was assumed that thermo-chemical signal was the sum of the signals of the single reaction steps. NETZSCH Kinetics Neo[®] has been the software used to analyze thermo-chemical processes data.

Preparation of functionalized polymers as chemical scaffolds.

In a typical Diels-Alder polymerization reaction, either the difurfuryl monomer **1** or **2** and the bismaleimide DMDOO (**3**) were dissolved in the polymerization solvent (mixtures of THF-H₂O with variable water content: 0%, 10% or 20% v/v) so that the maximum monomers concentrations were reached (concentration for monomer **1** or **2** and monomer **3**: 65 mM). The temperature was set at 20 °C, 30 °C or 40 °C by means of a silicone oil bath and the polymerization reaction was stirred for 48 hours. The reaction was quenched by freezing the reaction mixture at -20 °C. The solvents were removed under vacuum at low temperature and the bulk polymers further dried at high vacuum for 7 days, leading to the polymeric material in yields above 95% in all cases. Purification steps were unnecessary since unreacted monomers were not found, and the reactions proceeded without the formation of side products. All the samples were studied by ¹H NMR to determine not only the number molecular weight but also the segments' ratios *endo-exo*. In all cases, the presence of *endo* and *exo* adducts segments were found with prevalence of the former. From every experiment and after 24-hour polymerization time, an aliquot was removed from the reaction mixture and analyzed by ATR-FTIR to determine the percentage of monomer conversion.

Preparation of DiT-Fur-based Polymers: Type-A Materials. In the preparation of a batch of Type-A polymeric materials, ten polymerization reactions devised to optimize the polymeric conditions were conducted, as recorded in Table S1 (ESI), following the general method described above. All the polymers derived from DiT-Fur (**1**) were white solids.

FTIR-ATR: ν (cm⁻¹) 3309 N-H, 1684 C=O; 1257 δ C-N.

¹H NMR (CDCl₃, 500 MHz): δ (ppm) 7.35 (bs, 1H, H_a from terminal furfuryl group), 6.53 (bs, 4H, H_b and H_c *exo*), 6.42 and 6.33 (2 bs, 4H, H_b and H_c *endo*), 5.71 (bs, 2H, NH), 5.29-5.26 (m, 2H, H_a *endo*), 5.22 (bs, 2H, H_a *exo*), 4.34 (bs, 4H, H_f), 4.04-3.80 (m, 4H, H_e *endo/exo*), 3.70-3.40 (m, 14H, H_h, H_i *endo*), 3.35-3.20 (m, 2H, H_i *endo*), 3.10-2.85 (m, 6H, H_g, H_i *exo*, H_j *exo*). ¹³C NMR (CDCl₃, 125 MHz): δ (ppm) 175.9, 175.1, 174.7, 170.7 (C=O amide), 156.4 (C=O urethane), 138.5, 137.3 (C_b, C_c *exo*), 135.8, 135.1 (C_b, C_c *endo*), 90.2 (C_d), (80.8 (C_a *exo*), 79.4 (C_a *endo*), 70.1, 69.9, 68.0, 67.8, 67.1, 67.0, 38.3, 37.2 (C_h), 62.9 (C_f), 50.2 (C_i *exo*), 48.2 (C_i *endo*, C_i *exo*), 43.8 (C_i *endo*), 42.0 (C_e *exo*), 40.3 (C_e *endo*), 37.9 (C_g) (see ESI).

Preparation of DTT-Fur-based Polymers: Type-B Materials. With a similar strategy for the materials prepared from monomer **1**, ten polymerization reactions between DTT-Fur (**2**) and DMDOO (**3**) were carried out with success in agreement with the experimental parameters shown in Table S1 (ESI). In this case, the polymers derived from DTT-Fur (**2**) were syrup-like materials.

FTIR-ATR: ν (cm⁻¹) 3339 O-H, N-H, 1694 C=O; 1530 N-C=O st sy (amide II), 1244 δ C-N.

¹H NMR (DMSO-*d*₆, 500 MHz): δ (ppm) 8.34 and 8.28 (2 bs, 2H, NH), 7.58 (bs, 1H, H_a from terminal furfuryl group), 6.57, 6.41 and 6.27 (3 bs, 4H, H_b and H_c *endo* and *exo* isomers), 5.25 (bs,

2H, H_a *endo*), 5.10 (bs, 2H, H_a *exo*), 4.84 (bs, 2H, OH), 4.42-2.71 (m, 26H, H_e, H_f, H_g, H_h, H_i, H_j). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ (ppm) 176.5, 175.5, 175.2, 175.0 (C=O amide), 167.3, 166.7 (C=O thiourethane), 138.1, 135.9, 135.7 (C_b, C_c *endo* and *exo* isomers), 91.2, 90.9 (C_d *endo* and *exo* isomers), 80.6 (C_a *exo*), 78.9 (C_a *endo*), 70.1, 69.8, 69.7, 67.4, 66.8, 38.1, 37.6 (C_h), 72.0 (C_f), 50.5, 48.7, 48.2, 47.6 (C_i, C_i *endo* and *exo* isomers), 41.4, (C_e *exo*), 40.3 (C_e *endo*), 32.8 (C_g) (see ESI).

From quantitative ¹H-NMR analyses, the values of *M_n* were calculated and ranged 6,700 to 22,500, depending on the polymerization conditions. Likewise, experimental *endo/exo* segment ratios were determined and they varied between 74/26 and 45/55.

Experimental design for the optimization of polymerization conditions.

To disclose optimized polymerization conditions for the diene monomers, **1** and **2**, a Box-Behnken experimental design (CSS Statistica, StatSoft Inc., Tulsa, UK) was used to evaluate the significance of the independent variables (water content and temperature), as well as the interactions among them in the polymerization with the bisdienophile **3**.

The number of experiments “*N*” necessary was defined by the Equation (3):

$$N = k^2 + k + cp \quad (\text{Eq. 3})$$

where “*k*” represents the number of factors (variables) involved in the study and “*cp*” is the number of replicates of the central point. Box-Behnken could be seen as a cube, consisting of a central point and the middle points of the edges.

Thus, the influence of water content and temperature in the final molecular weight of the polymers was studied by means of 20 polymerization systems, 10 of them named **DiT-W_x-T_y** and the other 10 systems named **DTT-W_x-T_y**. They were prepared from monomers DiT-Fur (**1**) or DTT-Fur (**2**), respectively. In Table S1 (ESI document) and along the text “*x*” denotes the water content in percentage (v/v) in the polymerization solvent and “*y*” denotes the polymerization temperature (Celsius degrees). The bisdienophile *exo* was DMDOO (**3**) in all polymerization systems, the initial monomer concentration was set at 65 mM led to proceed for 48 h. The factorial design was used for monomer DiT-Fur (**1**) and DTT-Fur (**2**) in which number average molecular weight (*M_n*) was stated as dependent variable. The total number of experiments required for each system considered independent variables at three levels was 10. The values of the selected pair of independent variables were normalized from -1 to +1 by using Equation (4) to facilitate direct comparison of the coefficients and visualization of the effects of the individual independent variables on the response variable.

$$X_n = \frac{x - \bar{x}}{(x_{\max} - x_{\min})/2} \quad (\text{Eq. 4})$$

where “*X_n*” is the normalized value of independent variables; “*x*” is the absolute experimental value of the variable

concerned; " \bar{X} " is the mean of all fixed values for the variable in question; and " X_{max} " and " X_{min} " are the maximum and minimum values of the variable, respectively.

The data, analyzed by multiple regression analyses following polynomial equation, were derived to represent M_n as a function of the independent variables tested [Equation (5)],

$$y = \sum_{i=1 \text{ to } 3} \beta_i x_i + \sum_{i < j} \sum_{i=1 \text{ to } 3} \beta_{ij} x_i x_j + \sum_{i=1 \text{ to } 3} \beta_i x_i^2 \quad (\text{Eq. 5})$$

where " y " is the predicted M_n , " β ", " β_i ", and " β_{ij} ", denotes the regression coefficients and " x_i ", " x_j " are the normalized values between pairs of independent variables (water content and temperature). Only the estimated coefficients with significant levels higher than 95% ($p < 0.05$) were included in the final models. The differences between the experimental values and those that were calculated using the previous equations never exceeded 5% of the former.

Determination of monomer conversion in polymerization trials by Infrared Spectroscopy and a Second Derivative Procedure.

Infrared spectra were recorded with a Jasco FT/IR 4200 spectrometer equipped with ATR. FTIR spectra were collected in a wavenumber range of $4,000 \text{ cm}^{-1}$ to 600 cm^{-1} at a resolution of 4 cm^{-1} . To enhance the signal-to-noise ratio, 64 scans were co-added and signal averaged. The background reference material used was air. To improve the spectral features, the second derivatives of the absorption spectra were generated, and multiplied by -1 with the sole objective that the bands point upwards for convenience.

To study the percentage of monomer conversion at reaction time of 24 hours, the height of the band at 3098 cm^{-1} — associated with the stretching band of =C-H maleimide bonds— was measured in the negative second-derivative absorption spectra. These values diminished unparallelly with the monomer conversion. The band height was monitored in each polymerization trial and compared to selected reference standards. Among the latter, the value found at 0 hour for the polymerization media were linked to 0% of conversion. The figures associated with 100 % conversion were the heights of the peaks found when polymeric systems with number average molecular weights (M_n) above 17,500 determined by ^1H NMR (conversion degree ≥ 98) were utilized. The reduction of these data in percentage were correlated with the degree of conversion at selected times.

Results and discussion

Preparation of monomers and small molecules.

For the present study, the functionalized bisdifurfuryl monomers DiT-Fur (**1**) and DTT-Fur (**2**) were freshly prepared by reaction of furfuryl isocyanate with either 2,2'-dithiodiethanol (DiT) or D,L-dithiothreitol DTT, respectively. In both cases, the presence of difurfuryl urea as a side product was found. The interest of both monomers in biomedical formulations is due to the fact that monomer **1** imparts degradability under the hypoxic environments characteristic in solid tumor tissues;²⁹

monomer **2**, with two free hydroxyl groups per molecule, will increase the hydrophilic nature of the final polymer. The bisdienophile chosen for the current work, DMDOO (**3**), was prepared by an already published method.¹⁶ This bismaleimide also displays a hydrophilic character due to the short oligoethylene glycol chain that connects both maleimide rings (Fig. 1). To anticipate the thermal lability of the polymers to prepare, a model compound based on the bismaleimide monomer was synthesized. DMDOO monomer was made to react with furan to render molecule DMDOO-Fur (**4**) in almost quantitative yields.

Thermal stability studies of the model DA adduct DMDOO-Fur (**4**). Kinetic parameters determination under model-based analyses.

The total reversibility of furan-maleimide-based adducts seems to occur from $100 \text{ }^\circ\text{C}$ to $120 \text{ }^\circ\text{C}$, mainly at the upper values of the range.^{1,11,17,30,31} This effect has been used to provide with self-healing properties to thermo-labile materials at temperatures close to $120 \text{ }^\circ\text{C}$.¹⁷ Moreover, at lower temperatures, the retro-DA reaction has been observed. For instance, at $60 \text{ }^\circ\text{C}$ this reaction was significant when long reaction times (for example, 24 h) were associated.³⁰ Other authors have stated that at $70 \text{ }^\circ\text{C}$,³¹ furan-maleimide adducts were partially reversible and, at higher temperatures, short periods of time were needed (at $80 \text{ }^\circ\text{C}$ in 2 hours⁻¹) to isolate the products from the retro-DA reaction.

Some studies related to the thermo-reversibility of furan-maleimide DA adducts have been published. Thus, for example, Sanyal and coworkers prepared polymethacrylates with protected maleimide functionalization to deblock the maleimide units upon heating so that they would later participate in the preparation of hydrogel micro-pattern by Michael addition. They followed the reversibility of the Diels-Alder reaction by NMR at $80 \text{ }^\circ\text{C}$ and found that, upon heating for 30, 60 and 120 minutes, the conversion of the cycloadduct to maleimide was 18, 36 and 56%, respectively, while heating for 8 h ensures complete conversion with no trace of the bicyclic moiety.³² The particular nature of each DA reaction has already been demonstrated by the extensive work carried out by the group of Prof. Konovalov from Kazan Federal University and several predictive models have been assessed for calculating kinetic parameters of cycloaddition reactions.³³ Among the cycloaddition reactions studied (1,849), most of them corresponded to DA reactions. Each DA reaction (and consequently, its counterpart retro-DA) is, therefore, unique.

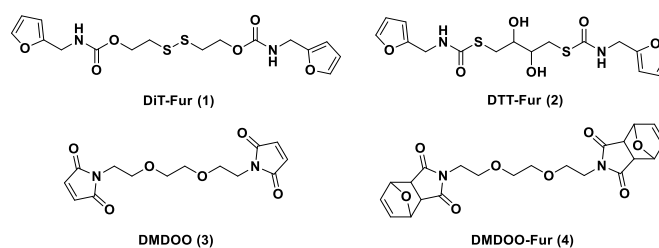


Fig. 1 Chemical structure of the monomers and small molecules used in the present study.

Given the above and that the polymer materials to be synthesized are based on DMDOO monomer, it is necessary to carry out a deep study of the influence of temperature on the stability of furan–maleimide adduct based on DMDOO.

To study the effect of temperature on the reversibility of the Diels-Alder adduct, a series of thermogravimetric trials were conducted on the model compound DMDOO-Fur (**4**) (Scheme 1). Compound **4** provided some interesting features: first, DMDOO was the bismaleimide to be used in the polymerization experiments; secondly, the degradation of adduct **4** would release the highly volatile furan (boiling point: 32 °C/758 mmHg; vapor pressure: 493 mmHg at 20 °C. Data from www.sigmaaldrich.com) and, consequently, the kinetics of the procedure could be unequivocally linked to the weight loss observed during the trials; third, thermogravimetric analyses are able to detect minute loss of weight (changes of a few thousandths of a milligram can be measured).

Figure 2 displays the thermograms obtained when compound **4** was heated under inert atmosphere at 50 °C, 60 °C and 80 °C for 100 min.

Surprisingly, at time periods as short as 100 min and temperatures as low as 50 °C, a non-negligible weight loss of 3.81% was observed. The latter value was associated with the deprotection of 12.5 % of maleimide groups present in the sample, rising to values of 24.4% and 49.3% at 60 °C and 80 °C, respectively. Certainly, the choice of temperatures to be used in the polymerization trials is by no means trivial.

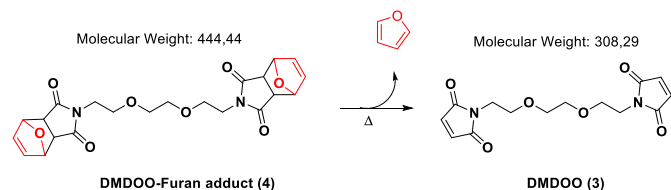
Likewise, a set of experiments were designed to calculate the kinetic parameters involved in the retro-DA reaction by TGA. The use of compound **4** provided relevant information on the expected thermo-lability of the polymer chains due to the similarity found between their labile segments and therefore, their degradability. Figure 3 discloses the thermal behavior of compound **4** when it was exposed to various heating rates between 50 °C and 135 °C.

As can be seen in Figure 3, the thermograms displayed similar profiles being remarkable the increase in degradability at low heating rates. For this reaction, the model scheme that has offered the best R^2 is the model in which one step $A \rightarrow B$ is applied. In that form, the utilized balance equations were Equation 6 and 7:

$$\text{Mass} = \text{Initial}_{\text{mass}} - \text{Total}_{\text{MassChange}} * \int \left(\frac{d(A \rightarrow B)}{dt} \right) dt \quad (\text{Eq. 6})$$

$$\frac{d(A \rightarrow B)}{dx} = A T^n e^{-\left(\frac{E_a}{RT}\right)} \quad (\text{Eq. 7})$$

After 10,000 iterations with different reaction orders, the better results ($R^2 = 0.973$) found have been: E_a = Activation Energy:



Scheme 1 Scheme of thermally-induced retro-Diels-Alder reaction of adduct 4.

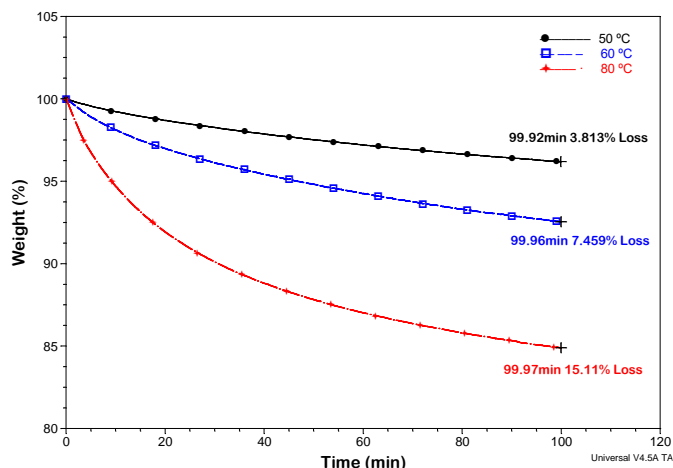


Fig. 2 Thermogravimetric profiles for thermally-induced weight loss of DMDOO-furan adduct 4 at various fixed temperatures under inert atmosphere.

136.16 kJ/mol; $A = \text{Log}(A): 17.674 \text{ Log}(1/s)$; n = Reaction Order: 3.71.

Synthesis of the new functionalized polymers as chemical scaffolds

As stated above, temperature is a crucial parameter in DA reactions. Diels-Alder reactions have been conducted at temperatures ranging from 20 °C to 60 °C.^{1,10,19,30} Besides, in some cases and starting from protected maleimide adduct as monomer, the DA coupling took place in parallel with the maleimide deprotection procedure and with controlled cooling rates from 120 °C to rt.²¹ In our case and based on the findings gathered in the thermogravimetric experiments of compound **4**, the selected polymerization temperatures used were 20 °C, 30 °C and 40 °C.

Regarding the polymerization solvents, we have recently demonstrated, like other authors, that the presence of water in the media during the reactions between maleimide and furan derivatives in hydrogel formations can bear a marked and positive effect on the kinetics of the coupling reaction between these moieties.^{16,34} There are many potential advantages of

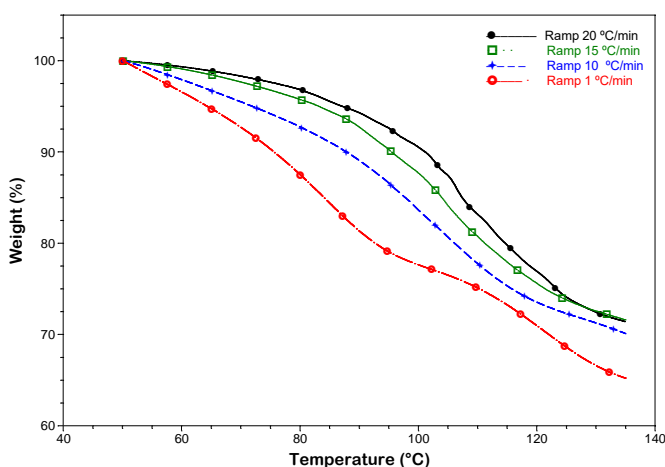


Fig. 3 Thermogravimetric profiles for thermally-induced weight loss of DMDOO-furan adduct 4 at various heating rates under inert atmosphere.

replacing non-natural solvents with water. The most obvious is the cost, since there is not any cheaper solvent than water; secondly, in aqueous media, the risks associated with organic solvents such as their carcinogenic, explosive, and/or flammable are features are avoided; thirdly, water is an eco-friendly solvent that can contribute dramatically to reduce the environmental pollution linked to the chemical industry. Accordingly, the development of non-hazardous alternatives is of great importance.²⁵ In addition, water displays some unique properties such as a large cohesive energy density, a high surface tension, and a hydrophobic effect that may be responsible not only for the “on water” effect, but also for the rate and selectivity enhancement of DA reaction. The enhanced hydrogen bonding and enforced hydrophobic interactions are also believed to play an important role in the rate accelerations of DA reactions in water.^{35,36}

Considering the above-mentioned benefits linked to the use of water, water was selected as a solvent in the current studies. However, the expected insolubility of the final polymeric materials in the media made mandatory the use of a cosolvent. Thus, the polymerization conditions were chosen so that the cease of polymerization reactions would be prevented. Due to the low solubility shown by DMDOO in most water-miscible solvents, tetrahydrofuran (THF) and mixtures of THF-H₂O (percentages of water by volume: 0%, 10% and 20%) were chosen to carry out the DA polymerization reactions (solubility of DMDOO in the polymerization solvents = 65 mM).

Twenty polymerization systems, 10 of them named DiT-W_x-T_y (Type A polymers: from **A1** to **A10**) and the other 10 systems named DTT-W_x-T_y (Type-B polymers: from **B1** to **B10**) were prepared from monomers DiT-Fur (**1**) or DTT-Fur (**2**),

respectively. In Table S1 (ESI document) and along the text “x” denotes the water content in percentage (v/v) in the polymerization solvent and “y” denotes the polymerization temperature (Celsius degrees). The bisdienophile used was DMDOO (**3**) in the 20 polymerization systems.

The thermal lability of maleimide-furan adducts at low temperatures, which, a priori, is a significant drawback for DA-materials in other fields, could be of great benefit in the preparation of smart devices with biomedical applications. Thus, for example, the thermal degradability of DA-based drug-carriers could be the key parameter for the controlled release of drugs at physiological temperatures. In addition, a further boost in drug release can be achieved by producing local hyperthermia by ultrasound.³⁷ Apart from their thermal lability at low temperatures, the presence of disulfide linkages in Type-A polymers, sensitive to the reduced glutathione (GSH) over-expressed in cancer tissues,²⁹ is an extra bonus in the development of new anticancer formulations. Multihydroxy type-B polymers, with hydrophilic segments capable of promoting hydrogen bondings, can exhibit mucoadhesive properties as outlined by Peppas and Huang,³⁸ with significant relevance in the development of gastroretentive drug delivery systems (GRDDS).³⁹

The initial monomer concentration was set at 65 mM and the polymerizations were led to proceed for 48 h and analyzed at 24 h by FTIR-ATR. The final molecular weights of the polymers were determined by quantitative end-group ¹H NMR analyses. Figure 4 records the ¹H NMR spectra of monomer DMDOO (**3**) and polymer DiT-W₀-T₃₀ in CDCl₃. The *endo* and the *exo* adducts in the polymer chains (Figure 4) give rise to different and well differentiated NMR peaks. Assignment of the signals to both

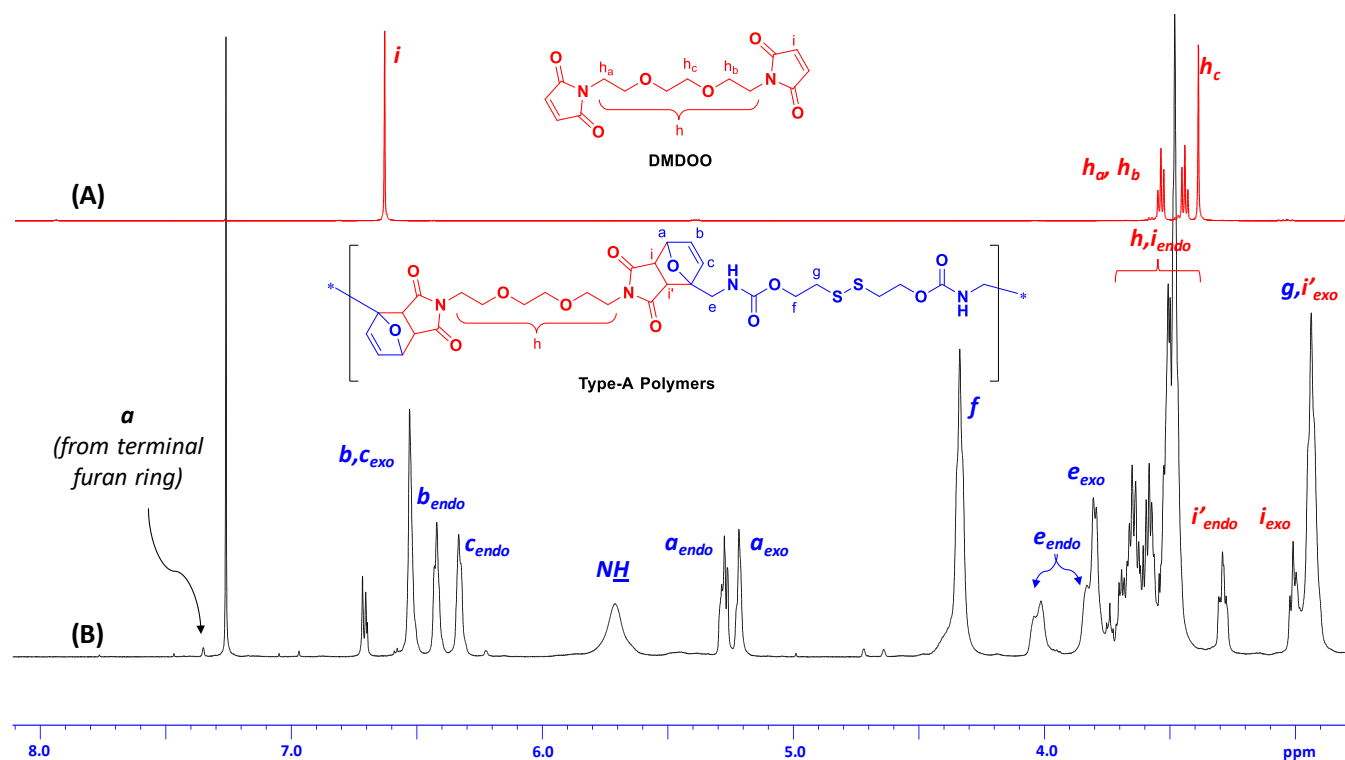


Fig. 4 ¹H NMR spectra (CDCl₃, 500 MHz) of (A) Monomer DMDOO (upper plot) and (b) the Type-A polymer DiT-W₀-T₃₀ (bottom plot).

adducts was based on COSY spectra and supported by other NMR data reported in the literature.¹⁶ Interpretation of the spectra began with the identification of “b” and “c” protons of both isomeric structures and correlating them unequivocally with “a” protons. The chemical shifts of the other protons in the polymer materials could then be easily deduced.

Assuming an equimolar consumption of maleimide groups with furfuryl moieties during the polymerization processes and the presence of one furan ring out of the two ends of every polymer chain, the weighted ratio between the integrals from protons “f” from the polymer at δ 4.34 ppm and proton “a” in the furan terminal group at δ 7.35 ppm rendered the average degrees of polymerization (\overline{DP}) of DiT-Fur-based polymers (Type-A polymers, Table 1) and, therefore, the number average molecular weights (M_n) of the synthesized materials (ESI, Equations S1a, and S2b). For the DTT-Fur-based polymers (Type-B polymers, Table 2) derived from monomers **2** and **3**, the signals used to calculate the \overline{DP} were the peaks related to protons “b” from the polymer at δ 5.26 and δ 5.11 ppm and the peak correlated with proton “a” from the terminal furan ring at δ 7.58 ppm (ESI, Equations S1b and S2).

Selected samples were also analyzed by GPC. It was observed that, in general terms, the M_n values obtained by GPC were magnified compared to those values calculated from quantitative ¹H NMR. Thus, for example, the trial that rendered the highest M_n for the Type-A polymers according to ¹H NMR ($M_n = 25,300$, sample **A3**, DiT-W₀-T₄₀) displayed the following values in the GPC chromatogram: $M_n = 42,200$; $M_w = 65,300$; $M_w/M_n = 1.6$ (Figure S1, ESI). A similar behavior was observed for DTT-based polymers and the values accessed from GPC were overestimated. Thus, for example, sample **B4** (DTT-W₁₀-T₂₀) with a calculated $M_n = 16,900$ from NMR data, displayed the following results from its GPC chromatogram: $M_n = 64,600$; $M_w = 95,500$; $M_w/M_n = 1.5$ (Figure S2, ESI). This effect may be due to the formation of hydrogen bonds between the urethane, hydroxyl and/or the thiourethane groups when the samples are

dissolved in the GPC solvent (DMF) causing some reversible chain connections and, therefore, an increase in the hydrodynamic volume of the polymer samples.

The estimation of the *endo/exo* ratios in the polymeric materials by ¹H NMR was straightforward. In the case of Type-A polymers and Type-B polymers, the molar *endo/exo* ratios were calculated according to Equation S3a and Equation S3b (ESI), respectively. One of the benefits linked to the use of water as a (co)solvent in DA reactions is its capacity of boosting reaction selectivity towards the *endo* isomer. Thus, *endo/exo* ratios have been almost invariably higher in water than in organic solvents.⁴⁰ This fact has been observed even when the reactants are sparingly soluble or insoluble in this medium.³⁵ Density functional theory studies endorse these findings since the computed *endo* preference is increased in aqueous media up to 2.4 Kcal mol⁻¹.⁴¹

It needs highlighting that the formation of the *endo* adducts prevailed over the *exo* adducts in almost all the polymerization trials (Table 1 and 2). This agrees with the Alder rule,⁴² which predicts the predominance of the *endo* stereoisomer when the furan/maleimide DA reaction is carried out at low to moderate reaction temperature.⁴³ When higher temperatures were set for the polymerization experiments, it was confirmed a general trend towards a slight but steady reduction in the *endo/exo* ratios. This concurs with the findings that the *endo* configuration is kinetically favored over the *exo* configuration whereas the latter is thermodynamically more stable.⁴⁴ The exception was observed in sample **B7** (DTT-W₁₀-T₄₀) with an *endo/exo* ratio close to 1:1 (45:55).

Determination of monomer conversion during polymerization by Infrared Spectroscopy and a Second Derivative Procedure.

Another target of the present work was to explore the possibility of monitoring the polymerization trials with a straightforward and affordable IR-based procedure that takes advantage of the fact that the required equipment is present in

Table 1 Type-A Polymers: Extent of polymerizations of DiT-Fur (1) and DMDOO (3) under different polymerization conditions obtained from IR and NMR data at 24 h and 48 h, respectively.

Polymerization trials		Polymerization conditions		FTIR-ATR data Polymeriz. Time: 24 h	¹ H NMR Data Polymerization Time: 48 h	
Formulation code	Sample	Water content (%)	Temperature (°C)	Functional groups consumed (%)	Molar ratio <i>endo/exo</i>	M_n
DiT-W ₀ -T ₂₀	A1	0	20	50	68/32	13200
DiT-W ₀ -T ₃₀	A2	0	30	57	62/38	22000
DiT-W ₀ -T ₄₀	A3	0	40	62	56/44	25300
DiT-W ₁₀ -T ₂₀	A4	10	20	71	62/38	4900
DiT-W ₁₀ -T ₃₀	A5	10	30	80	66/34	10700
DiT-W ₁₀ -T ₃₀	A6	10	30	78	66/34	11300
DiT-W ₁₀ -T ₄₀	A7	10	40	86	62/38	11400
DiT-W ₂₀ -T ₂₀	A8	20	20	65	66/34	8900
DiT-W ₂₀ -T ₃₀	A9	20	30	*	60/40	15800
DiT-W ₂₀ -T ₄₀	A10	20	40	*	55/45	18200

[Bis-maleimide monomer] = [Difurfuryl monomer] = 65 mM; polymerization time: 48 h.

Solvent: THF with variable water content (in %v/v): 0%, 10% or 20%. **Temperature:** 20 °C, 30 °C, 40 °C.

* Signal-to-noise ratio (SNR) < 10

Table 2 Type-B Polymers: Extent of polymerizations of DTT-Fur (2) and DMDOO (3) under different polymerization conditions obtained from IR and NMR data at 24 h and 48 h, respectively.

Polymerization trials		Polymerization conditions		FTIR-ATR data	¹ H NMR Data	
Formulation code	Sample	Water content (%)	Temperature (°C)	Polymeriz. Time: 24 h	Polymerization Time: 48 h	
				Functional groups consumed (%)	Molar ratio endo/exo	M _n
DTT-W ₀ -T ₂₀	B1	0	20	55	74/26	22500
DTT-W ₀ -T ₃₀	B2	0	30	46	53/47	9000
DTT-W ₀ -T ₄₀	B3	0	40	65	59/41	6700
DTT-W ₁₀ -T ₂₀	B4	10	20	67	70/30	16900
DTT-W ₁₀ -T ₃₀	B5	10	30	80	66/34	12500
DTT-W ₁₀ -T ₄₀	B6	10	30	*	56/44	13000
DTT-W ₁₀ -T ₄₀	B7	10	40	*	45/55	7600
DTT-W ₂₀ -T ₂₀	B8	20	20	*	71/29	16800
DTT-W ₂₀ -T ₃₀	B9	20	30	*	64/36	15400
DTT-W ₂₀ -T ₄₀	B10	20	40	*	56/44	14600

[Bis-maleimide monomer] = [Difurfuryl monomer] = 65 mM; polymerization time: 48 h.

Solvent: THF with variable water content (in %v/v): 0%, 10% or 20%. **Temperature:** 20 °C, 30 °C, 40 °C.

* Signal-to-noise ratio (SNR) < 10

most research laboratories. In addition, the procedure for testing polymerization reactions is minimally invasive (removing a small aliquot from the medium for analysis is sufficient), and it is not necessary to be extremely cautious when taking out the aliquot since the reaction is not sensitive neither to oxygen, nor to the presence of water.

Consequently, IR spectroscopy was used as a supporting tool to track the polymerization processes after 24 h of reaction. These studies were conducted using attenuated total reflection (ATR)—Fourier Transform (FT) IR spectroscopy. To maximize broad convergence between the designed polymer systems and thus, enable the comparison between them, polymerization trials shared the same bismaleimide monomer (DMDOO, **3**) and the difurfuryl monomers have identical molecular weights (400 Da for DiT-Fur (**1**) and DTT-Fur (**2**); Figure 1). Likewise, identical monomer concentrations and reaction conditions were used for the preparation of type-A and type-B materials.

The degree of polymerization was calculated by means of quantifying the unreacted maleimide functional groups from monomer DMDOO (**3**). The focus was placed on the stretching band at ν_{\max} 3098 cm^{-1} associated with its $\text{Csp}^2\text{—H}$ bonds (Figure 5). The enhanced sensitivity of the negative second derivatives of the absorption IR spectra was used to determine the unreacted maleimide groups present in the reaction mixture. Thus, for example, the peak height found at polymerization time = 0 was set as the reference value for the presence of 100% unreacted maleimide monomer.

Polymerization trials were analyzed at 0 h, 7 h, 24 h and 48 h. In all samples, the peak at ν_{\max} 3098 cm^{-1} was indistinguishable from the baseline of the IR spectrum after 48 hours. For illustrative purpose, Figure 6 shows the second derivative of the IR absorbance spectra of the polymerization essay **A1** at 0 h, and at 48 h. However, this stretching band was still evident in most experiments after 24 h of polymerization.

To establish the range of monomer consumption that can be accurately measured with this technique, a minimum signal-to-

noise ratio (SNR) of 10/1 was set. Data from polymerization trial **A7** (DiT-W₁₀-T₄₀) at 24 h were used. The baseline noise for the second derivative spectrum was found to be 0.002 milliabsorbance unit/ cm^{-2} , while the negative second derivative band height observed for the peak at ν_{\max} 3098 cm^{-1} was 0.022 mAU/ cm^{-2} (signal-to-noise ratio = 11/1). The percentage of maleimide groups consumed was then quantified by ¹H NMR and found to be 86%. This data was correlated with the significant lower height of the selected IR band. Consequently, for the experiments conducted, monomer consumptions ranging from 0% to 86% could be provided by this method.

All the polymerization tests of the current work (**A1-A10** and **B1-B10**) were analyzed by IR at 24 hours. The heights of the second derivative of their absorption bands were measured. The conversion degrees were calculated by means of the reduction in intensity observed of the band mentioned above

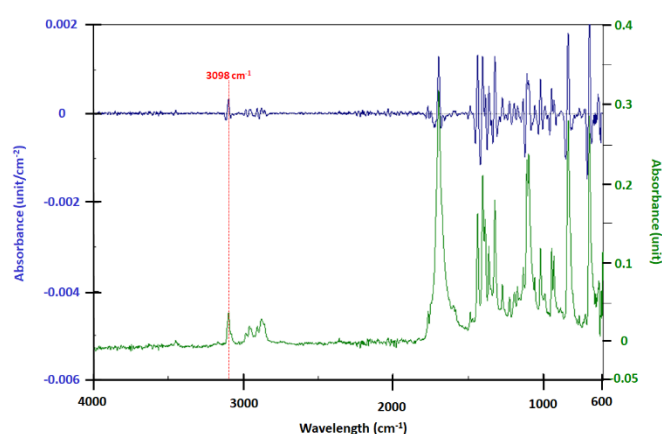


Fig. 5 The absorbance spectrum (bottom) and its corresponding negative second derivative spectrum (top) for the bismaleimide monomer DMDOO (**3**). A vertical line indicates the position of the unique band due to C—H bonds in maleimide rings at wavenumber (ν_{\max}) 3098 cm^{-1} .

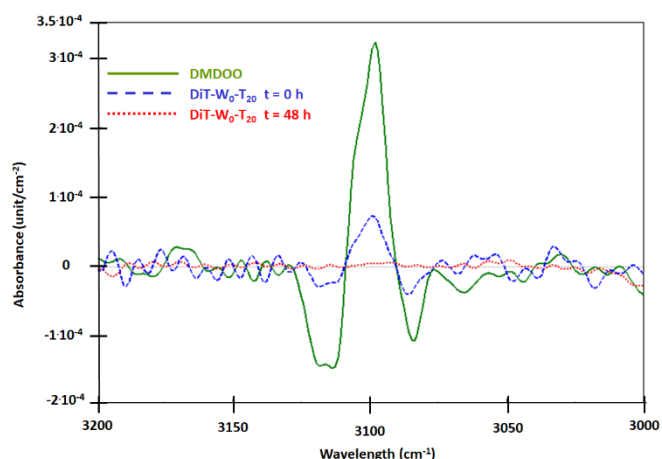


Fig. 6 Expanded spectral region that exhibits the negative second derivatives of the vinylic C-H stretching band from maleimide rings at $\nu_{\max} = 3098 \text{ cm}^{-1}$ (Solid green line: DMDOO monomer; blue dash line: polymerization trial **A1** (DiT- W_0 - T_{20}) at time = 0 h; red dotted line: polymerization trial **A1** (DiT- W_0 - T_{20}) at time = 48 h).

(data recorded in Tables 1 and 2). This signal tended to vanish gradually as the polymerization proceeded.

Type-A polymers exhibited monomer consumptions larger than 50% in all cases (Table 1). Temperature played a significant role speeding the polymerization reactions likewise the increment of water content in the polymerization media. Thus, for samples **A9** and **A10**, the peak at $\nu_{\max} 3098 \text{ cm}^{-1}$ was undetectable after 24 h of reaction. Regarding Type-B polymers, they displayed the same trend that Type-A polymers when the solvent was THF. However, those trials with water as cosolvent were quicker than their counterparts, with monomer consumptions bigger than 80% in 24 hours. This may be due to the presence of hydroxyl groups in the bisdiene monomer **2** that may enhance the solubility of the oligomers in the reaction media.

All in all, considering the affordable nature of the required equipment and that this technique is minimally invasive (the removal of a small aliquot for analysis is sufficient), quantitative IR analyses are highly convenient to determine in what extent DA polymerizations have taken place.

Experimental design for the optimization of polymerization conditions.

One of the main goals of the current work is to draw the optimal conditions for conducting the polymerization of the chosen bisfuranyl monomers **1** and **2** with bismaleimide **3** under mild conditions. To disclose optimized polymerization conditions for the diene monomers, **1** and **2**, a Box–Behnken experimental design was utilized and the election of two independent variables was required. The independent variables were

chosen, temperature and solvent, ranged from 20 °C to 40 °C and from 0% to 20% of water content, respectively. As dependent variable, M_n , calculated by $^1\text{H NMR}$, was selected. Table 1 and 2 display the values of independent variables and the experimental values of M_n obtained for the polymerization of DMDOO (**3**) with both bisfuranyl-based monomers: DiT-Fur (**1**) and DTT-Fur (**2**), respectively. Both tables also content the information about *endo/exo* ratios found in each trial. In Table 3, the equations obtained using polynomial regression and statistical parameters (R^2 , *df* and *F*) are shown.

Concerning the response equation, in most cases an acceptable R^2 (>0.90) and *F* (>29) values have been found. Both equations contain complex terms that involve interactions between the independent variables. Identifying the influence of the relative independent statistical variables on the dependent variable in the displayed equations is not straightforward, nor are the calculations to obtain the two values of independent variables at which the maximum M_n could be achieved. To overcome this drawback, the response surfaces for the dependent variable in each system studied are shown in Figure 7A as well as the comparative influence of the independent variables in M_n in DTT-based systems (Fig. 7B) and DiT systems (Fig. 7C).

M_n for Type-A Polymers were calculated and ranged from 4,900 to 25,300, depending on the polymerization conditions (Table 1). The experimental *endo/exo* segment ratios were calculated and were found to be from 68/32 to 55/45. In the case of Type-B Polymers, M_n values were within 6,700 and 22,500, figures also depending on the polymerization conditions (Table 2). Likewise, experimental *endo/exo* segment ratios were determined and they varied between 74/26 and 45/55. It could be observed that both polymerization systems (Type-A polymers and Type-B polymers) rendered similar ranges of M_n but with different behaviors when the polymerization conditions vary as is described in detail below.

The most relevant findings were that the correlations of M_n with the independent variables (temperature and water content) were marked dissimilar for both polymerization systems (Type-A and Type-B polymerizations), being temperature, the most influential parameter as can be seen in Figure 5A, 5B and 5C. Surprisingly, the influence of temperature was divergent in the two systems studied. Thus, for DiT-Fur based polymers (from **A1** to **A10**), temperature played a positive influence (increments in molecular weights) over the entire temperature range, whereas a consistent decrease linked to temperature in the final M_n was observed in DTT-Fur polymers (**B1**–**B10**). This fact can be easily brought to front when comparing trials conducted at equal water content, i.e., trials **B1**, **B2** and **B3**. Reductions in degree of polymerizations linked to moderate temperatures were partially compensated when water was added to the systems

Table 3 Equation yielded for the dependent variable (M_n) as a function of the independent variables (water content and temperature, normalized values) for the experimental design.

Equation	R^2	<i>df</i>	<i>F</i>
$DiT - M_n = 10,900 - 2,933.33 W + 4,650 T + 8,100 W^2 - 2650 T^2$	0.98	4.5	51.77
$DTT - M_n = 13,500 - 1,433.33 W - 4,550 T + 3,400 WT$	0.95	5.40	29.33

W = water content, normalized value; T = Temperature, normalized value; M_n = Number Average Molecular Weight determined by end-group analyses (from $^1\text{H NMR}$) for the polymerization of DMDOO with (a) DiT-Fur (DiT- M_n) and (b) DTT-Fur (DTT- M_n).

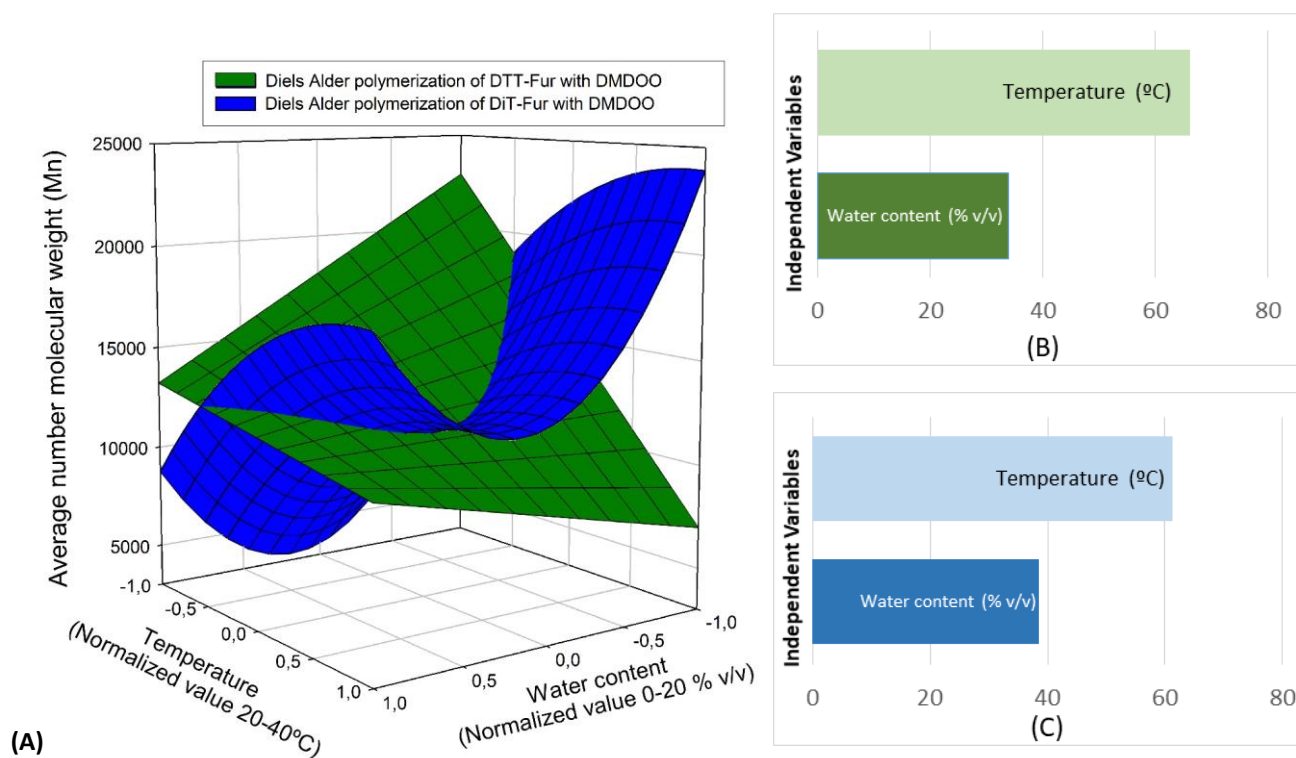


Fig. 7 Left column: (A) Response surface for Number Average Molecular Weight (M_n) (calculated by $^1\text{H NMR}$) against temperature and water content for the experimental design of the polymerization of DMDOO and DTT-Fur (green response surface) DMDOO and DiT-Fur (blue response surface). Right column: Relative influence (in percentages) of independent variables in Diels Alder Polymerization of (B) DTT-Fur with DMDOO (green) and (C) DiT-Fur with DMDOO (blue).

(for example, **B9** and **B6** compared to **B2**). On the other hand, in Type-A trials, water content displayed a non-linear behavior, leading to the highest M_n in the absence of water. However, the minimum M_n figures were found at medium water content (10% in volume). From the response surfaces for the dependent variable in the two systems studied (Figure 7A), similar degrees of polymerizations were achieved at intermediate temperatures and water contents (crossing line between curves).

It is clear that there is not a direct correlation between reaction rates and M_n . Other factors such as the reversibility of the reaction at moderate temperatures and accessibility of the final polymer chain to keep the polymerization process active need being considered. Thus, for example, from our own results described in Section 3.4 and also supported by other works,^{20,43} temperature increased the DA rates in all cases. However, this trend did not necessarily lead to polymers with high molecular weights. Based on the thermal lability of DMDOO-Fur (**4**) demonstrated in Section 3.2, the new polymeric materials are expected to be sensitive to intermediate temperatures, experiencing retro-DA reactions in some degree. Therefore, although high temperatures can speed up DA reactions, retro-DA processes are present, and need being introduced in the final equation. This could be the case of Type-B polymers, which displayed a trend towards low M_n at moderate temperatures. Regarding water content, it was assumed that the access to terminal functional groups in polymer chains was an essential requisite for keeping the polymerization process active. The use of water as cosolvent might interfere the growing of the polymer chains. This effect may be responsible for the reduction

in M_n of DiT-based polymers, due to the poor solvation of the oligomers generated during the polymerization process. These observations are also responsible for a peculiar behavior of some polymeric surfactants.⁴⁵ The lack of water solubility of the hydrophobic block from the mentioned polymeric surfactants was crucial for their irreversible thermal deactivation in which the polar and non-polar segments were initially joined by a furan/maleimide DA adduct. Consequently, the solvation of the final material in the reaction medium should have a key role in keeping active the DA polymerization.

To sum up, for Type-A polymers the best polymeric conditions were found at 40 °C when THF was used as solvent, whereas for the Type-B polymerizations, low temperature was critical to render higher M_n .

Thermal properties of the new materials.

The thermal properties of selected polymeric materials were characterized by DSC and TGA and are summarized in Table 4. Two samples from each family of polymers were analyzed to find (di)similarities among materials that belongs to the same or to different type of polymers.

The thermal degradability of the polymers was studied by thermogravimetric analyses (TGA) between 20 °C and 700 °C. Although retro-DA decomposition stage occurs during TG experiments, a weight loss is not always linked to it since nonvolatile oligomers may be formed. On the other hand, other thermally-induced decomposition stages may be present. The TG profiles (weight loss vs. temperature) of batch A showed a two-stage degradation process whereas the B series was

Table 4 Thermal properties of selected DiT-Fur and DTT-Fur based polymers.

Polymer		DSC ^a			TGA ^b		
		T _g (°C)	°T _{retro-DA} (°C)	maxT _{retro-DA} (°C)	°T _d (°C)	maxT _d (°C)	ΔW (%)
A3	DiT-W ₀ -T ₄₀	54.2	95.2	111.7	235	272/418	42/35
A4	DiT-W ₁₀ -T ₂₀	54.2	84.7	105.0	218	259/411	52/34
B4	DTT-W ₁₀ -T ₂₀	--	98.9	109.8	124	88/177/266/417	8/14/35/32
B9	DTT-W ₂₀ -T ₃₀	31.9	91.5	109.0	173	106/181/276/415	7/10/33/31

^aGlass transition temperature (T_g), onset retro-DA temperature (°T_{retro-DA}) and maximum rate retro-DA reaction temperature (maxT_{retro-DA}) measured by DSC. ^bOnset decomposition temperature corresponding to 10% of weight loss (°T_d), maximum rate decomposition temperatures (maxT_d) and weight loss at the respective decomposition step [ΔW(%)] determined by TGA.

degraded by a multistage procedure, the latter series being markedly more sensitive to heat than the former. This fact could give us a clue as to why the polymerization of DTT monomer (**2**) with DMDOO (**3**) proceeded to higher *M_n* when temperatures lowered.

From DSC analyses, glass transitions (T_g) took place at well-defined temperatures, with values that were highly dependent on polymer constitution. DiT-based polymers (series A) and DTT-based polymers (series B) exhibited an amorphous structure with T_gs of 54 °C and 32 °C, respectively.

Additionally, and from previous works, we have observed that in hydrogels cross-linked by maleimide-furan adducts, the gel-sol transitions, calculated from modulated temperature differential scanning calorimetry (MTDSC) experiments, were found to be in the range of temperatures comprised between 86.0 °C to 94.5 °C, depending on the copolymer composition.¹⁶ This range dovetails well with the temperature range in which the onset of the retro-DA reaction was observed in the materials prepared according to DSC data (from 84.7 °C to 95.2 °C, See Table 4). The maximum rates of the retro-DA were accomplished in a very narrow range of temperatures (from 105 °C to 111.7 °C), which fits well with that published by other authors.^{17,46}

Conclusions

The optimal conditions for the Diels Alder (DA) polymerization of bisfurfuryl monomers DiT-Fur (**1**) with bismaleimide DMDOO (**3**) (Type A polymers) and DDT-Fur (**2**) with bismaleimide DMDOO (**3**) (Type B polymers) regarding the two independent variables: temperature (from 20 °C to 40 °C) and water content in THF as a (co)solvent (from 0% to 20% in volume), have been determined in the present work. The experimental model design proposed herein has demonstrated to be a highly efficient and straightforward method to optimize the polymerization conditions of the DA reactions studied. It was found that temperature was the most influential variable in both systems, with the temperature range used in the experimental design selected after conducting a set of thermo-degradability trials. The furan-protected DMDOO-Fur (**4**) was the molecule of choice to study the influence of temperature on the stability of furan–maleimide adducts by thermogravimetric analyses and hence, to have a first approach to the thermo-degradability of the new materials. When exposed to various

heating rates from 1 °C/min to 20 °C/min, molecule **4** remarkably experienced the retro-DA reaction, and hence degradation, at temperatures as low as 50 °C. Consequently, temperatures below 50 °C were chosen for the polymerization trials.

Over the range studied, the maximum *M_n* attained for both Type A and Type B polymers was comparable. For Type A polymers, the optimal polymerization conditions were found in the absence of water and at 40 °C, the highest temperature used. Conversely, for the Type B polymers, obtained from the more hydrophilic DTT-Fur (**2**), temperature displayed a negative effect on final *M_n*, achieving the higher *M_n* when the water content was greater.

Additionally, an efficient and economical alternative to NMR end-group analyses has been designed to enable the determination of DA conversions in the initial stages. The improved sensitivity of the second derivative of IR absorbance spectra led to the development of this reliable IR-based method that allows the straightforward determination of the extent of polymerization during the reaction. Conversions were determined based on the height of the band at ν 3098 cm⁻¹ associated with C-H bonds in maleimide rings. This approach permitted the accurate determination of unreacted maleimide groups from the initial content at time = 0 min (100%) to a content of 14%, based on the limit of detection found for the method.

From calorimetric studies it was found that maximum rates of the retro-DA reaction in the polymeric materials were experienced in a very narrow temperature range (from 105 °C to ca. 112 °C), which supports that published by other authors.^{1,11,17,30,31}

Using the experimental design has provided significant benefits in the preparation of these tailor-made responsive polymers. The ability to rapidly and easily discover the optimal polymerization conditions for the preparation of the functional polymers, along with a novel method to track the polymerization, has greatly enhanced the development of these biomaterials. We anticipate that this approach can draw the attention of the scientific community and extend to other systems in which key independent variables exert a marked influence in the final product.

Conflicts of interest

There are no conflicts to declare.

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