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Simultaneous Measurement of Fluorescence, Conversion and Physical/mechanical Properties for Monitoring Bulk and Localized Photopolymerization Reactions in Heterogeneous Systems

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Abstract

An FT-NIR spectrometer, rheometer and fluorescence spectrophotometer were coupled for the real-time monitoring of polymerization reactions, allowing the simultaneous tracking of polymerization kinetics, storage modulus as well as fluorescence. In this study, a methacrylate functionalized dansyl chromophore (DANSMA) was synthesized and two different nanogels were made from urethane dimethacrylate and isobornyl methacrylate. Two series of resin formulations were prepared using the DANSMA probe, ethoxylated bisphenol A dimethacrylate as the matrix monomer, Irgacure[®] 651 as the initiator and the dispersed, monomer-swollen nanogels to give clear UV-curable resins. Placement of the fluorescent probe either throughout the resin or linked into the nanogel before its dispersion in the matrix provides a tool to study how the nanogel structure affects local network development by means of fluorescence from the DANSMA probe. We demonstrate the potential of this new technique using a composite as the two phase system (resin and polymerizable nanogel) including a dansyl derivative as a polymerizable probe to follow the reactions that are taking places in both phases.

Abstract

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Introduction

The design of improved polymeric materials is facilitated through a better understanding of the network formation process. Controlling polymerization kinetics is important to achieve the desired network structure and physical properties. Nanostructured materials, due to their very large surface area, are ideal additives for tuning networks due to their high degree of interactions. In the case of nanogel additives, the surface effects are compounded with the effects of monomer infiltrated within the nanoparticles. These polymeric materials reinforced with nanogel additives show enhancements in strength as well as reductions in polymerization shrinkage and stress, desirable in many applications including dental materials.^{1, 2} Previous studies have demonstrated the effects of nanogels as additives to model dental monomer and composite formulations that can potentially deliver enhanced clinical outcomes.³⁻⁵

We have prepared polymeric nanogels that can be swollen by monomers in order to tune final network structure and bulk properties. The nanogel additives achieve this control in part by influencing reaction kinetics in their immediate environment.⁶ Polymerization kinetics can be followed using NIR, but it is mainly a bulk analysis technique and cannot probe the local kinetics in and around the nanogels.⁷ Fluorescence probe methods are very useful for monitoring processes in polymers because of their high specificity, selectivity and rapid response time. In addition, probes can be used for in situ measurements. The fluorescent response of chromophores has been shown to detect changes in the local environment due to their sensitivity to factors such as polarity and viscosity.⁸

Monomer reaction data from other instruments like photo-DSC or FT-IR combined with fluorescent probe analysis can distinguish the different steps involved in a polymerization mechanism at the microscopic level, which cannot be observed only with calorimetric or spectroscopic measurements.⁹ In the same way, physical and mechanical properties can be correlated with conversion by coupling FT-IR spectroscopy and dynamic mechanical techniques, such as tensometer,¹⁰ dynamic mechanical analyzer¹¹ or rheometry.¹² This work represents the first time that we can examine changes and heterogeneities at the sub-

microscopic level by augmenting an FT-NIR analytical approach with fluorescence spectroscopy.

Herein, we report the coupling of rheology and fluorescence spectroscopy with NIR to study the polymerization behaviour of nanogels dispersed in a resin composite. This provides new insights into the time-dependent correlation between microscopic viscoelastic properties and polymerization kinetics in different areas of the composite materials: inside and outside the nanogel additive, in this example.

Experimental section

Materials

For the chromophore synthesis, dansyl chloride (>99%), 2-aminoethyl methacrylate hydrochloride (97%), triethylamine (>99%), ethyl acetate (99.5%), dichloromethane (99.5%, dried over 4 Å molecular sieves) from Sigma-Aldrich were used as received unless specified otherwise.

For the nanogel syntheses and resin formulations, isobornyl methacrylate (IBMA, 98.5%), 2-mercaptoethanol (ME), azobisisobutyronitrile (AIBN, 98%), ethoxylated bisphenol-A dimethacrylate (BisEMA, >98%) and ethyl acetate (99.5%) were obtained from Sigma-Aldrich. Urethane dimethacrylate (UDMA) was obtained from Esstech. 2,2-Dimethoxy-1,2-diphenylethane-1-one (Irgacure® 651) was purchased from BASF (Ciba-Geigy). The inhibitor was removed from IBMA by treatment with activated basic alumina (Brockman I, Sigma-Aldrich). AIBN was purified by recrystallization from methanol (m.p. 104 °C) before use and dried under vacuum at room temperature. All other materials were used as received.

Flash column chromatography was performed using silica gel (60 Å pore size, 230-400 mesh particle size, Merck). For the synthesis of *N*-dansyl-2-aminoethyl methacrylate, the reaction was monitored using thin layer chromatography (TLC) on silica gel-coated plates (Merck 60 F254). Detection was performed with UV light.

Methods

¹H NMR and ¹³C NMR spectra were recorded on a Bruker 300 MHz spectrometer with samples dissolved in CDCl₃ at room temperature. Chemical shifts were assigned using the residual undeuterated solvent signal as an internal reference.

Elemental analyses were made with a LECO CHNS-932 apparatus.

Mass spectra were recorded using an HPLC-MSD 1100 mass spectrometer (MS) with an atmospheric pressure ionization source or an electrospray ionization (ESI) source.

Triple-detection Gel Permeation Chromatography (GPC; Viscotek) with differential Refractive Index (RI, Viscotek VE 3580), viscosity and light scattering detectors (Viscotek 270 dual detector) was employed for the analysis of nanogel weight average molecular weight (M_w), number average molecular weight (M_n), polydispersity index (PDI) and average hydrodynamic radius (R_h). The mobile phase was HPLC grade THF/Acetone (90:10)

v/v) at a flow rate of 1.0 mL/min at 35 °C in a series of four columns spanning molecular weight of 10^4 – 10^7 Da. Poly(methyl methacrylate) standards were used for calibration.

Fourier transform near-infrared (FT-NIR) spectra were recorded using a *Thermo Scientific Nicolet 6700* spectrophotometer with a resolution of 4 cm^{-1} . The kinetics of the photopolymerization were monitored using real-time FTIR spectroscopy equipped with two fiber optic cables (Multimode Fiber Optics) through a NIR fiber port (*Smart Fiberport, Nicolet Instrument*). These fibers feature pure fused silica fiber cores (1 mm diameter), numerical aperture of 0.22 mm, a wide wavelength range (350–2400 nm) and transmission efficiency higher than 90%.

The samples were exposed simultaneously to UV light (*EXFO Acticure 4000* mercury arc lamp using a 365 nm narrow bandpass filter with light intensity on the surface of the sample measured in mW/cm^2 using an EIT radiometer) which induces the polymerization, and to a NIR beam, which provides for *in situ* analysis of the extent of the reaction. Reaction progress was followed by monitoring the methacrylate group conversion by the real-time decrease in the absorbance of the $=\text{CH}_2$ first overtone absorption band centered at 6165 cm^{-1} as the monomer is converted to polymer. The methacrylate group conversion is calculated according to the following formula:

$$\text{Conversion} \quad (t) = (1 - A_t/A_0) \times 100$$

Where A_0 is the initial area of the absorbance peak related to the methacrylate double bond of the monomer and A_t is the peak area measured at different irradiation times.

Volumetric shrinkage was measured using a linometer (ACTA, Academic Center for Dentistry, Amsterdam, The Netherlands), as described previously.¹³ Briefly, a drop of monomer held between a free aluminum disc and a fixed glass slide was irradiated with the resulting displacement of the disc and the differences in potential monitored by a Linear Variable Differential Transformer.

The linometer was equipped with an *EXFO Acticure 4000* mercury arc lamp ($\lambda = 365 \pm 10\text{ nm}$) and coupled to FTIR (*Thermo Scientific Nicolet 6700* spectrophotometer equipped with $100\text{ }\mu\text{m}$ diameter NIR fiber optic cables) that provides measurement of volumetric shrinkage and methacrylate conversion simultaneously. The NIR source was directed through the center of the sample and conversion was calculated using the same approach as in the kinetic analysis. Incident UV light irradiance was $25\text{ mW}/\text{cm}^2$ for 10 min in all experiments. Three replicates were conducted for each system.

Post-gel polymerization stress development was monitored in real-time under ambient conditions using a cantilever beam-based tensometer (Volpe Research Center, American Dental Association Health Foundation, Gaithersburg, MD). As described previously,¹⁴ a monomer sample is placed between two quartz rods, which are attached to the fixed base of the apparatus and to a deformable cantilever beam. As polymerization proceeds, the polymer bonds to the silane methacrylate surface treated rod ends and deflects the beam with stress then calculated based on the cross-sectional area of the specimen and a calibration curve of

the beam constant obtained previously. The rate of stress development (R_s) was calculated as the first derivative of the stress versus time curve.

The tensometer was also equipped with an *EXFO Acticure 4000* mercury arc lamp ($\lambda = 365 \pm 10$ nm) that provided UV activation of the photopolymerization using one of the quartz rods as a light guide. The sample was probed by FTIR (*Thermo Scientific Nicolet 6700* spectrophotometer equipped with NIR fiber optic cables), which allows the shrinkage stress data to be coupled directly with methacrylate conversion. The details of this instrument and its operation are described more completely in other publications.^{15, 16} All samples were disc-shaped, with 6 mm diameter and 1 mm thickness. Incident UV light irradiance was 1 mW/cm² for 30 min in all experiments. Three replicates were conducted for each material.

For the rheology tests, a rheometer (*ARES TA Instruments*) was used to measure the initial viscosities and the dynamic evolution of storage modulus for nanogel modified resins with 8 or 20 mm diameter parallel plates at 20 °C, 5 rad/s frequency and 50% constant strain (ensuring that the test was carried out within the linear viscoelastic regime). Three replicates were conducted for each system.

A chamber was constructed to allow nitrogen purging of all samples. Each sample underwent 30 min of N₂ purge before analysis with the plates separated 2 mm to remove dissolved oxygen and avoid oxygen-inhibited edge effects that otherwise confound the rheological data. Afterwards the two quartz plates were brought to a distance of 0.3 mm, creating a cylindrical sample volume of 0.3 mm thickness and 8 or 20 mm diameter depending on the sample.

Coupling Rheometer to FTIR and to Fluorimeter (Scheme 1)—In this inert atmosphere chamber a fluorescence detector *USB4000 Ocean Optics* (equipped with a Toshiba 3648 element CCD -charge coupled device- linear array detector to increase the signal/noise ratio while detecting photons across the 200–1100 nm range) was coupled through a 50 μ m optical fiber. It is of critical importance that this fiber is aligned horizontally with the sample and at an optimal 90-degree angle from the incident light source.

For following the polymerization reaction, changes in emission have been analysed in terms of the ratio between the fluorescence intensity at two different wavelengths (FI_2/FI_1), as discussed in previous papers.^{9, 17} The wavelengths used for the dansyl probe in this study were 495 nm (λ_{em1}) and 545 nm (λ_{em2}), which avoids interaction with the photocuring process.

The rheometer was also equipped with an *EXFO Acticure 4000* mercury arc lamp ($\lambda = 365 \pm 10$ nm) that was coupled to an in-house designed optical apparatus allowing both UV and NIR direct transmission access to specimens within the rheometer so storage modulus and methacrylate conversion are registered simultaneously.¹⁴ Methacrylate conversion was measured using a *Thermo Scientific Nicolet 6700* spectrophotometer equipped with near NIR fiber optic cables. The NIR source was directed through the center of the sample and

conversion was calculated using the same approach as in the kinetic analysis. Incident UV light irradiance was 1 mW/cm² for 30 min in all experiments.

Synthetic procedures

Synthesis of N-dansyl-2-aminoethyl methacrylate (DANSMA)—2-Aminoethyl methacrylate·HCl (280 mg, 1.70 mmol, 1 equiv.), triethylamine (1.1 mL, 7.65 mmol, 4.5 equiv.) and ethyl acetate (20 mL) were stirred at room temperature for 1 h. The white precipitated solid was separated by filtration. Then, a solution of dansyl chloride (550 mg, 2.04 mmol, 1.2 equiv.) dissolved in ethyl acetate (20 mL) was added drop-wise to the reaction mixture. The mixture was stirred at room temperature for 4 h. The volatiles were removed under vacuum and the crude product was purified by column chromatography on silica gel (dichloromethane/ethyl acetate, 12:1 v/v) giving DANSMA (462 mg, 75%) as a pure yellow-green powder.

¹H NMR (300 MHz, CDCl₃) δ ppm: 8.58 (d, H, *J* = 8.5 Hz), 8.26 (m, 2H), 7.55 (m, 2H), 7.19 (d, H, *J* = 7.3 Hz), 5.89 (s, H), 5.47 (s, H), 5.17 (t, H, *J* = 6.0 Hz), 4.06 (t, 2H, -NH-CH₂-CH₂-, *J* = 5.2 Hz), 3.22 (m, 2H, -NH-CH₂-CH₂-), 2.91 (s, 6H, N-(CH₃)₂), 1.79 (s, 3H, C-(CH₃)).

¹³C NMR (75 MHz, CDCl₃) δ ppm: 166.8, 151.5, 135.4, 134.2, 130.6, 129.8, 129.6, 129.5, 128.5, 126.2, 123.3, 118.9, 115.4, 62.8, 45.5, 42.4, 18.1.

Elemental analysis calcd for C₁₈H₂₂N₂O₄S: C, 59.65; H, 6.12; N, 7.73; S, 8.85. Found: C, 59.89; H, 6.31; N, 7.24; S, 8.19.

ESI-MS (*m/z*) calcd for C₁₈H₂₂N₂O₄S [M]⁺ 362.0; found [M+H]⁺ 363.0.

Syntheses of nanogels (Ng and NgDANS)—Nanogels were developed by following a slight modification of a literature strategy.¹⁸ Urethane dimethacrylate (UDMA, 2.0 g), isobornyl dimethacrylate (IBMA, 3.8 g), ethyl acetate (24 mL, a four-fold excess relative to monomers), 2-mercaptoethanol (ME, 0.13 g, 10 mol% relative to monomers) and azobisisobutyronitrile (AIBN, 28 g, 1 mol% relative to the IBMA monomer content). The solutions were heated in an oil bath for 3 h at 80 °C under a nitrogen atmosphere with a circulated water condenser in place.

After the reaction, the samples were analysed by FTIR spectroscopy, reaching more than 60% conversion. The clear nanogel reaction mixture was added dropwise to a 10-fold excess of methanol (400 mL), which resulted in the precipitation of the polymeric materials. The precipitate was isolated by decanting and the residual solvent was removed under high vacuum affording the nanogel (2.71 g, 72%). ¹H NMR characterization of the resulting nanogels demonstrated copolymer compositions similar to that of the monomer feed ratios. Since the nanogel synthesis was stopped below full conversion, there are some pendant reactive groups derived from UDMA that are located throughout the nanogel structure.

Two types of nanogels were synthesized: Control nanogel, designated as Ng and nanogel containing the chromophore, designated as NgDANS, in which DANSMA was added as a comonomer in the nanogel synthesis at a concentration of 0.03 mol% relative to the overall

monomer content. The reaction, isolation and characterization procedures were identical for the Ng and NgDANS materials.

Formulation of resins—Two different sets of formulations were prepared to obtain a broad range of formulated methacrylate resin system, which enable us to investigate the polymerization behaviour of nanogels dispersed to form a resin composite. Formulations in Series 1 include the fluorescent label DANSMA, as a polymerizable comonomer in the BisEMA resin while formulations in Series 2 introduce the fluorescent label covalently linked to the nanogel particles (Scheme 2). In both series of materials, the dansyl chromophore was included at very low concentration. Its presence as free comonomer did not alter the BisEMA reaction kinetics and similarly, no kinetic differences were noted for nanogel additives with or without the presence of the tethered probe.

Nanogels were added to the BisEMA resin in mass ratio of 5, 10, 15 and 20 wt%. Unfilled BisEMA without any of the dansyl probe (NgDANS0) was used as the control. *Irgacure 651* (0.1 wt%, relative to resin) was added to each sample. Samples were mechanically agitated (2-48 h) until all prepolymers were fully dispersed to give optically clear monomer/nanogel compositions.

Final resin compositions and their references are summarized in Table 1.

Results and discussion

Synthesis of N-dansyl-2-aminoethyl methacrylate (DANSMA)

The synthesis of the reactive dansyl probe followed a different synthetic strategy than the method reported recently.¹⁹ Here the synthesis was performed in a two-step one pot reaction. Methacrylate functionalization of dansyl chloride was carried out by liberation of 2-aminoethyl methacrylate from its hydrochloride, under mild conditions, and subsequent *in situ* acylation of the amino group with dansyl chloride at room temperature. The DANSMA chromophore was isolated with an overall yield of 75% (Scheme 3).

In this way, total reaction time is reduced from 24 h to 5 h, one purification step is avoided and the total yield is improved from 13% (overall two-step procedure as previously described in the literature) to 75%.

Syntheses of nanogels (Ng and NgDANS)

For the nanogel syntheses, UDMA and IBMA were mixed at 20:80 molar ratio and 2-mercaptoethanol was added as a chain transfer agent. Fine control of the mercaptoethanol concentration avoids macrogelation along with control of the average primary chain length as well as nanogel size and molecular weight. AIBN was added as the free radical thermal initiator. DANSMA was added at a concentration of 0.03 mol% relative to the monomer content. The overall process of nanogel synthesis is shown in Scheme 4.

GPC analysis demonstrated that both series of nanogels presented relatively broad molecular weight distributions as expected for the globular, highly branched ($M-H a < 0.5$), multi-chain

structures. Both nanogels present similar mean hydrodynamic radius results (~ 7 nm in THF). Specific GPC data are summarized in Table 2.

Ethoxylated bisphenol-A dimethacrylate monomer (BisEMA) was chosen as the photopolymerizable resin. It is widely used in dental restorative applications as well as in UV cured coatings due to its resistance to swelling and staining, flexibility, low shrinkage, high modulus and strength.² Monomer structures are shown in Scheme 5.

Formulation of resins

When nanogels are dispersed in resin of an appropriate solubility parameter, the internal free volume associated with the nanogel structure and solution polymerization process allows for significant swelling by monomer.¹⁸ Once this secondary photopolymerization reaction begins, the matrix monomers in the composite formulation polymerize outside and inside the nanogel particles including copolymerization with residual methacrylate groups in the nanogel. This provides both a physically interpenetrated polymer network as well as a covalent attachment between the nanogel and the matrix polymer (Scheme 6).

Considering the different formulations listed in Table 1, these could be represented in the four series as shown in Scheme 7. As particle-particle interactions become dominant above the nanogel percolation threshold, which is typically encountered at about 10 wt% loading depending on the nanogel size and swelling potential, the viscosity of resin formulations greatly increases with increasing nanogel content. In prior studies, TEGDMA formulations loaded up to 50 wt% with similar nanogels reach 100 Pa·s,¹⁸ while BisGMA monomer (specifically designed for use in dental materials) has monomeric viscosity values between 700 and 1300 Pa·s.²⁰ Viscosity values of the formulations studied in the present work are shown in Figure 1.

Viscosity increases with increasing nanogel content from 9 Pa·s for the neat BisEMA resin to almost 600 Pa·s for the formulation with 20% nanogel content. Without a reactive diluent comonomer such as TEGDMA, it was difficult to obtain homogeneous formulations without solvent dilution and subsequent removal when nanogel content exceeded 20 wt%.

Series 1 and 2 (Ng and NgDANS, respectively) presented similar viscosity values with the nanogel content due to their close similarity in terms of nanogel composition and size.

Photopolymerization kinetics

Photopolymerization kinetic profiles and polymerization rate plots for Series 2 (formulations with the fluorescent label linked to the nanogel particles) are shown in Figure 2.

The kinetic profiles show the conventional behaviour of a photocrosslinking curing reaction of a glassy polymer with autoacceleration in the initial stage of polymerization and final conversion values limited by vitrification.¹¹ During UV irradiation, good photostability of the chromophore was observed. The presence of the chromophore, whether freely dispersed or localized within the nanogel, does not affect the kinetics of the polymerization reaction relative to the chromophore-free analogue controls due to its low content in the mixture.

As expected with the addition of the high glass transition temperature nanogels (bulk nanogel $T_g \sim 90^\circ\text{C}$) to a moderately viscous monomer, progressive differences in rate of polymerization and limiting conversion values were observed as a function of nanogel content. Final conversion values decreased from 70% for BisEMA control to 63% for the formulation with 20% nanogel content. Also, a similar trend is found for both the initial and maximum rates of polymerization. In general, the increase in nanogel loading caused a decrease in the initial rate of polymerization and a delay in the maximum rate of polymerization. These effects are likely caused by the restricted environment for BisEMA monomer inside and around the nanogel particles. The viscosity of neat BisEMA has been shown to promote autoacceleration from the onset of polymerization.²¹ It is assumed that the monomer-swollen nanogel environment restricts monomer mobility and slows the polymerization reaction relative to the bulk resin. In prior studies with a lower viscosity monomer matrix such as TEGDMA, addition of nanogel increased the overall reaction rate with little effect on final conversion.¹⁸

Volumetric polymerization shrinkage

Volumetric shrinkage was measured in real-time during the photopolymerization process with a linometer. The degree of conversion of the shrinkage specimens was simultaneously monitored by transmission mode NIR spectroscopy via fiber optic cables. Plots obtained by linometer measurements are shown in Figure 3 and data are compiled in Table 3. The nanogel systems generated much lower shrinkage than the unfilled BisEMA. Volumetric polymerization shrinkage decreases in a relatively linear manner with increasing nanogel content with some indication that above the percolation threshold of $\sim 10\text{ wt\%}$, the degree of the shrinkage reduction is somewhat diminished. A prior study with a different nanogel/resin formulation showed progressive shrinkage reduction continuing to 40 wt% nanogel loading levels.²² Here, at equivalent mass fraction loading levels, the formulations with the chromophore dispersed in the resin (Ng) showed slightly lower values although not statistically significant differences as compared with formulations with the chromophore linked to the nanogel (NgDANS). In resin loaded with 20 wt% nanogel content (NgDANS20), the shrinkage was reduced by 35% approximately compared to the unfilled resin, with final shrinkage values around 3%.

As shown in Figure 4, the simultaneously acquired conversion/time and volumetric shrinkage/time plots can be coordinated to display shrinkage with respect to conversion, which provides a nonlinear relationship.

Correlation between volumetric shrinkage with conversion showed three stages: an initially low rate of shrinkage up to 20% conversion followed by an accelerating progression in shrinkage, which accounts for the majority of the shrinkage the material undergoes most of the shrinkage percentage. Finally, beyond approximately 60% conversion, shrinkage in the vitrified state is significantly suppressed relative to conversion. In the intermediate stage, formulations with low nanogel content (5 and 10%) behave similar to the unfilled control resin. However, when including 15 and 20% nanogel, where a more significant proportion of the resin matrix is located within the swollen nanogel particles, a delay in the onset of the more rapid shrinkage phase is observed above 30% conversion. The predominant

contribution to the volumetric polymerization shrinkage occurs during the deceleration phase of the polymerization reaction, which is reached at higher conversion for formulations with nanogel contents that are above the percolation threshold. This as well as the reduced overall reactive group concentration associated with the reactive nanogel additive as compared with the monomer lead to the lower shrinkage of the nanogel-modified resin.

Nearly all polymerizations involve some degree of shrinkage during polymer formation and these results in strain. In compositionally homogeneous materials, the static polymerization shrinkage obtained depends on the initial reactive group concentration and the degree of conversion achieved.^{23, 24} In our systems, the shrinkage of the formulations was significantly reduced with increasing nanogel fraction. Polymerization stress is related to the storage modulus and to the polymerization-induced strain,²⁵ so as polymerization shrinkage is reduced, there is potential for reduction in polymerization stress as well.

Polymerization stress

To examine the polymerization stress behaviour of our labelled systems, stress evolution during polymerization was monitored in real time using a tensometer while simultaneously recording the degree of conversion by NIR. For the BisEMA control, stress rapidly increased reaching a final stress value of about 5.8 MPa (Figure 5). By incorporation of 5% or 10% nanogel content in the monomer, significant progressive reductions in stress (12 and 41%, respectively) were achieved compared to the unfilled BisEMA control. In these formulations with relatively low nanogel content, the stress reduction was achieved without compromise in final conversion of the resulting polymers.

Photo-rheology study. Real-time and simultaneous monitoring of photopolymerization kinetics, storage modulus and fluorescence emission changes

Once confirmed that the photocuring conditions do not affect the chromophore beyond its copolymerization and that the macroscopic polymerization parameters do not appreciably vary when the fluorescent label is added to the system, either inside the nanogel particles or in the bulk of the photopolymerizable formulation, spectroscopic fluorescence monitoring was introduced as an additional detection technique in the system. To enable this, storage modulus was continuously measured in real-time during polymerization by conducting photopolymerization between the quartz plates of a photorheometer (Figure 6). In this way, the photorheometer was coupled with both NIR and fluorometric detection systems that allowed simultaneous irradiation of the sample, measurement of conversion and monitoring of fluorescence emission while also registering the evolution of the storage modulus. All of the above measured parameters, except fluorescence emission, are “macroscopic”, meaning they provide information averaged over the entire sample.

However, since the fluorescence probe is located in different spatial domains (inside the nanogel or in the bulk of the resin), the measurement of fluorescence emission will give information only over the microenvironment of the probe.

Both series 1 and 2 formulations showed similar development of mechanical properties during the photopolymerization reaction (Figure 7). Final storage modulus values were very similar, around 0.80-0.85 GPa, independent of nanogel content. In Figure 7.b the variation of

storage modulus as a function of conversion is shown only for the series 2 materials. The slight initial increase in storage modulus can be better appreciated on the logarithmic axis, showing the property development profile that includes macrogelation before 5% conversion based on the crossover between the loss and storage moduli (not shown here but previously demonstrated for nanogel/resin systems¹²). The equivalent linear data better shows the dramatic increase in storage modulus at the end of the irradiation process, which corresponds with vitrification.

For all the formulations under study here, Figure 8 shows representative polymerization profiles recorded by FT-NIR and by fluorescence spectroscopy. Resin-labelled formulations are shown on the left whereas the analogous nanogel-labelled formulations are shown on the right side.

Before irradiation, a higher emission intensity ratio value is observed when the probe is linked to the nanogel particles (NgDANS), which indicates an initial higher rigidity around the probe located exclusively inside the nanogel. This behaviour is explicitly shown in Figure 9. The initial fluorescence value of FI_2/FI_1 is approximately the same independent of nanogel content in the sample, which is logical as the only variation is the nanogel content in the system and the fluorescence ratio is not dependent on the chromophore concentration.

As irradiation takes place, the evolution of the reaction is perfectly followed by fluorescence emission of the probe. When DANSMA is dispersed in the resin (Figures 8.a, 8.c, 8.e and 8.g on the left), kinetic profiles obtained by fluorescence are identical to the FTIR conversion plot throughout the entire polymerization range. When the probe is located exclusively inside the nanogel (Figures 8.b, 8.d, 8.f and 8.g on the right), these profiles are different as the probe is not sensing the bulk but the variations occurring due to polymerization in and around the nanogel particles. Thereby, at the beginning of the polymerization, in all cases, the fluorescence emission intensity ratio decreases during the first seconds of irradiation, which indicates a temporary decrease in rigidity inside the nanogel particles. This may be related to a physical expansion of the nanogel as contraction of the bulk matrix begins. This also could be coupled with some degree of monomer diffusion in or out of the nanogel driven by the localized changes in free volume or conversion differential. During autoacceleration (expanded inset plots), the delay in polymerization inside the nanogel particles is shown clearly, as well as a slower rate of polymerization based on the diverged signals up to 40-50% conversion depending on nanogel content. At higher conversions, behaviours inside and outside the nanogel are similar. These differences found between the bulk and the nanogel during the photopolymerization reaction are more notable with the addition of higher nanogel content to the formulation as might be expected since the fraction of monomer infused in the nanogel particles increases with nanogel loading.

When monitored by FTIR, the kinetics of polymerization are similar in the systems loaded with the same nanogel content. However, kinetic profiles obtained from fluorescence monitoring showed two different behaviours when the probe is linked to the nanogel or dispersed in the resin.

Figure 10 shows, as an example, the two different behaviours observed during the photopolymerization, where the considerable delay in the degree of double bond conversion inside the nanogel and the higher overall rigidity associated with the nanogel domain throughout the polymerization are highlighted.

The changes in fluorescence intensity ratio of the probe have been correlated with the storage modulus (Figure 11). The response of the probe shows a parallelism but different situations inside and outside nanogel particles. As shown above, a higher emission intensity ratio value is observed when the probe is linked to the nanogel particles (NgDANS). At higher conversions, fluorescence behaviour is identical inside and outside the nanogel although shifted in terms of the intensity ratio. However, at lower conversions, a more compressed increase in (FI_2/FI_1) is observed for the nanogel-bound probe. This fact, together with the different kinetic behaviour observed in this polymerization range, suggests that the differences between loaded and unloaded systems are mostly produced at this stage of polymerization.

These preliminary results are very promising. Since conventional techniques used to study the polymerization process provide information about the bulk or the macroscopic system, labelling with fluorescent probes in different phases of a heterogeneous system could allow the detailed study of the potentially complex processes that take place in each phase.

Conclusions

The fluorescent probe DANSMA has been included in a resin composite in two different ways, either chemically attached to the nanogel or dispersed in the matrix monomer BisEMA. The probe showed intense fluorescence emission, which varies in intensity and in the maximum wavelength of emission during the photopolymerization reaction. This provides the possibility to study *in situ* and in real time how the polymerization kinetics are locally affected by the nanogel and its structure as the microstructural differences in the networks formed are responsible of the changes in the fluorescence spectra. These changes have been simultaneously monitored and correlated with photopolymerization kinetics and with the mechanical data obtained by photo-rheology study.

While conventional techniques to study polymerization processes give average information about the macroscopic system, fluorescence is sensitive to the microenvironment of the probe and this allowed us to distinguish two different behaviours inside the nanogel and in the matrix resin. These results are promising since the use of fluorescent probes in the different phases of a composite material could allow study of the processes that take place in each of them.

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Figure 1.
Initial viscosity values for NgDANS0-20 resin formulations before photopolymerization.
Note: error bars are included but are generally smaller than the symbols.

Figure 2.

a) Kinetic profile and b) Instantaneous rate of polymerization (R_p) as a function of conversion for Series 2 formulations (NgDANS0-20). Irradiated with 365 nm UV light at 25 mW/cm² for 10 min.

Figure 3.

Volumetric polymerization shrinkage as a function of (a) irradiation time and (b) the nanogel content (at the end of the polymerization). Samples irradiated with 365 nm UV light at 25 mW/cm² for 10 min.

Figure 4. Volumetric polymerization shrinkage of Control and NgDANS5-20 resins as a function of conversion degree. Samples irradiated with 365 nm UV light at 25 mW/cm² for 10 min.

Figure 5.

Polymerization stress in BisEMA monomer and resin formulations with 5 and 10% nanogel content. Irradiated with 365 nm UV light at 1 mW/cm² for 30 min.

Figure 6. Photographs of the in-house customized design coupling the photorheometer (1), FT-NIR (2) and fluorescence detection system (3) is shown. Prior to the plates being set to the desired gap, the open sample (4) is purged under nitrogen inside a chamber (5) followed by irradiation with 365 nm UV light (6).

Figure 7.

Storage modulus (G') during photopolymerization reaction of formulations with 0, 5, 10, 15 and 20 wt% nanogel content vs. a) irradiation time and b) conversion. Prior to polymerization, the viscosity effect associated with nanogel loading levels is reflected in the higher initial G' values. Samples were irradiated with 365 nm UV light at 1 mW/cm² for 30 min. Identical G' data are plotted on either log (left axis) or linear (right axis) scales.

Figure 8.

Variation of fluorescence (emission intensity ratio, $\lambda_{em}(1) = 495$ nm, $\lambda_{em}(2) = 545$ nm) and reaction conversion with irradiation time during photopolymerization reaction of formulations with 5, 10, 15 and 20% nanogel content. Samples irradiated with 365 nm UV light at 1 mW/cm^2 for 30 min.

Figure 9.
Initial values of FI_2/FI_1 before irradiation for all the different nanogel contents.

Figure 10. Variation of fluorescence intensity ratio with conversion for the formulation with 5% nanogel content. Samples irradiated with 365 nm UV light at 1 mW/cm² for 30 min.

Figure 11.

Variation of storage modulus with fluorescence intensity ratio ($\lambda_{em}(1) = 495$ nm, $\lambda_{em}(2) = 545$ nm) during the photopolymerization of formulation with 5, 10, 15 and 20% nanogel content. Samples irradiated with 365 nm UV light at 1 mW/cm^2 for 30 min.

Scheme 1.

Experimental system used in the connection among Rheometer (1), IR (2) and Fluorimeter (3). Sample (4) is previously purge under nitrogen inside a chamber (5) and it is irradiated with 365 nm UV light (6).

Series 1 **Series 2** **Scheme 2.**

Series 1 and 2 of the prepared formulations.

Scheme 3.
Synthesis of DANSMA chromophore.

Scheme 4.

Reaction description for the nanogel synthesis of labelled nanogels.

Urethane dimethacrylate (UDMA)

Isobornyl methacrylate (IBMA)

Ethoxylated bisphenol-A dimethacrylate (BisEMA)

Scheme 5.
Molecular structures for methacrylate monomers.

Scheme 6.

Graphical representation of resin with nanogel formulations where nanogels are swollen with monomer resin before photopolymerization and “IPN and copolymer formation” is shown in the transition to the final polymer. Dispersed nanogel is shown representing low nanogel loading but a continuous nanogel-based phase would result above the percolation threshold.

Before polymerization

After polymerization

Ng0

Ng5-20

NgDANS0

NgDANS5-20

Scheme 7.

Graphical representation of the different studied systems depending on the composition. Blue color indicates the resin and orange color indicates DANSMA fluorescent label which can be located linked either in the bulk of the resin (Ng5-20) or only inside the nanogel particles (NgDANS5-20).

Table 1

Formulation of resins. Irgacure 651 (0.1 wt%) was added in each mixture. Unfilled BisEMA (NgDANS0) was used as the control experiment.

	Reference	BisEMA, wt%	NgDANS, wt%	Ng, wt%	DANSMA, wt%
Series 1	Ng0	100	0	0	0.01
	Ng5	95	0	5	0.01
	Ng10	90	0	10	0.01
	Ng15	85	0	15	0.01
	Ng20	80	0	20	0.01
Series 2	NgDANS0	100	0	0	0
	NgDANS5	95	5	0	0
	NgDANS10	90	10	0	0
	NgDANS15	85	15	0	0
	NgDANS20	80	20	0	0

Table 2

Reaction conversion, number and weight averaged molecular weights (Conv., M_n and M_w , respectively), polydispersity index (M_w/M_n), Mark-Houwink 'a' value and hydrodynamic radius (R_h) for the nanogels.

	Conv. (%)	M_n (g/mol)	M_w (g/mol)	M_w/M_n	MH-a	R_h (nm)
Ng	69	32,000	107,900	3.37	0.362	6.59
NgDANS	60	28,200	138,600	4.91	0.355	7.22

Table 3

Mean volumetric polymerization shrinkage values (%) of the composite resins after polymerization. Three replicates were conducted for each system.

Sample	Nanogel (wt%)	Volumetric shrinkage (%)
Ng0	0%	4.82 ± 0.17
NgDANS0		5.01 ± 0.23
Ng5	5%	-
NgDANS5		4.38 ± 0.11
Ng10	10%	3.36 ± 0.28
NgDANS10		3.80 ± 0.17
Ng15	15%	3.34 ± 0.23
NgDANS15		3.50 ± 0.31
Ng20	20%	3.16 ± 0.35
NgDANS20		3.24 ± 0.04