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Tugrul Cem Bicak, Maylis Garnier, Michèle Sabbah, Nébéwia Griffete. Photoinduced synthesis of fluorescent hydrogels without fluorescent monomers. *Chemical Communications*, 2022, 58 (69), pp.9614-9617. 10.1039/D2CC02888C . hal-04153241

HAL Id: hal-04153241

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Submitted on 6 Jul 2023

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COMMUNICATION

Fluorescent Monomer-Free Synthesis of Fluorescent Hydrogels in One Step by Type II Photoinitiated Polymerisation

Received 00th January 20xx,
Accepted 00th January 20xx

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DOI: 10.1039/x0xx00000x

A fluorescent monomer-free one-step strategy is developed for the synthesis of fluorescent acrylamide gels, using inexpensive and commercially available rhodamine B as the hydrogen donor in type II photoinitiation system. Hydrogels are highly fluorescent and have limited fluorophore leaching over time, due to the covalent bond formed between the polymer network and rhodamine dye.

Hydrogels are physically or chemically crosslinked three-dimensional networks that can highly swell in aqueous environment and they have been extensively investigated due to their applications in material science.¹ Traditional methods for hydrogel synthesis include photopolymerisation of vinyl monomers,² click reaction,³ Michael-type addition,⁴ and using Schiff base linkages.⁵

Fluorescent hydrogels, in particular, are of great importance due to their wide-range applications as sensors,⁶ imaging probes,⁷ and drug delivery.⁸ From a number of techniques available for the incorporation of fluorescence properties to the nanogels including the use of metal nanoparticles and quantum dots, the use of conjugated organic fluorescent monomers during polymerisation is still the most popular one due to their large molar extinction coefficients, specific spectroscopic properties, and electronic configurations.⁹

Despite the presence of alternatives to incorporate an organic fluorophore into the polymer network such as post functionalisation of precursor gels with a functionalised fluorophore,¹⁰ the use of a fluorescent monomer during polymerisation step is a commonly employed method to covalently bind fluorescent molecules to hydrogels.¹¹ Although, the method is easy and requires no additional step for functionalisation, fluorescent monomers are usually expensive due to their synthetic procedure. Moreover, the fluorescence intensity of the resulting gel is restricted by the

solubility of fluorescent vinyl monomers in the reaction medium - which is often water based.

Depending on the radical generation mechanism, photoinitiators are classified as type I and type II. Upon irradiation, type I photoinitiators undergo cleavage, whereas type II photoinitiation is realised *via* hydrogen abstraction from a donor molecule by a photosensitiser in its triplet excited state to form radicals that start polymerisation. Tertiary amines, alcohols, and ethers can be used as type II photoinitiators, and a wide range of electromagnetic spectrum (from 300 nm to 1000 nm) can be used for irradiation in type II photoinitiation, making their use suitable in combination with both heat and photo sensitive compounds.

The use of photoinitiation for hydrogel synthesis benefits from the inherent characteristics of the photoreactions such as spatial and temporal control by simply switching on and off the light source. In addition, photopolymerisations require less energy compared to their thermally initiated analogues since they can be carried out at room temperature. Type II photoinitiation has already been employed for hydrogel synthesis using a one-component photoinitiator¹² and using an acetylene functional initiator to incorporate click functionality into the polymer network in one step.¹³

Previously, we reported the first combined use of type II photoinitiated polymerisation with precipitation polymerisation for the synthesis of highly crosslinked microspheres at ambient temperature.¹⁴ Using an acetylene functional hydrogen donor in type II photoinitiation system, the method allowed in-situ acetylene functionalisation of the particles, enabling their modification via the copper(I)-catalyzed alkyne-azide cycloaddition reaction.

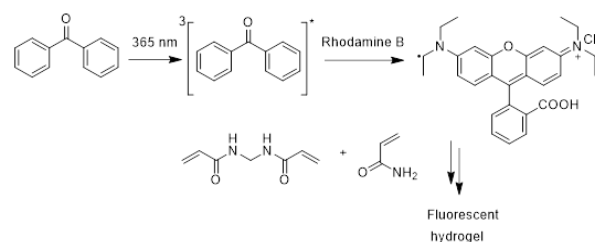


Fig. 1 The mechanism of hydrogel synthesis using RhB – BP bimolecular photoinitiation system.

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

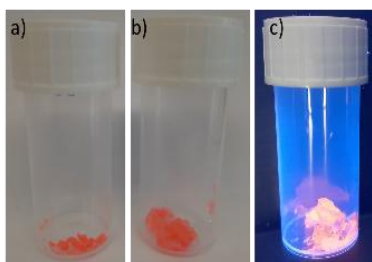


Fig. 2 Photograph of the hydrogels in their dry state (a), after 20 min in contact with water (b), and under UV irradiation after 20 min in contact with water (c).

Herein, we investigate the possibility to use, in one step, a water soluble Rhodamine B (RhB) as hydrogen donor in bimolecular photoinitiation system in combination with benzophenone (BP), as the photosensitizer molecule, to synthesise and in-situ functionalise hydrogels with RhB. This novel method allows a fast, simple and cheap synthesis of fluorescent hydrogels.

Type II photoinitiation is employed for hydrogel synthesis by simply replacing one component water soluble initiator with RhB – BP bimolecular initiation system as hydrogen donor and photosensitizer, respectively. Upon irradiation at 365 nm, BP is excited to its triplet state, and it abstracts hydrogen from the tertiary amine moiety of the RhB and finally generate radicals at the vicinity of the tertiary amine to start polymerisation. Since RhB is employed as photoinitiator in the current methodology, it is covalently linked to polymer network, paving the way for its fluorescent character with limited fluorophore leaching over time. The overall mechanism is illustrated in Fig. 1.

Table 1 summarises the reaction conditions employed for the hydrogel synthesis in this study. The proof of concept was tested by an initial experiment, which was carried out to investigate the possibility of incorporating RhB into the hydrogels in one step. The rate of polymerisation is slower for bimolecular photoinitiation compared to type I photoinitiators.¹⁵ Therefore, to ensure a successful polymerisation, an initial experiment was carried out using a concentrated monomer solution and long irradiation times (24 h) for polymerisation (Table 1, entry 1). Next, we investigated the possibility of obtaining nanosized and separate hydrogels using this method by simply optimising the reaction conditions (Table 1, entries 2-4). For this purpose, the total monomer concentration is set to 18 mg/ml and the photoinitiator to monomer ratio (mol%, with respect to total monomer) was gradually decreased (0.6:100, 0.3:100, and 0.1:100) while keeping other parameters such as monomer concentration, reaction temperature, and irradiation wavelength, the same.

Table 1 Reaction conditions employed for the hydrogel synthesis in this study

Entry	[Monomer]: [BP] mole ratio	Monomer Concentration (mg/ml)	Irradiation time (h)	Particle size (nm) ^a	Yield (%) ^b
1	[100]:[3]	50	24 h	Coagulum	54
2	[100]:[0.6]	18	6 h	197	41
3	[100]:[0.3]	18	24 h	200-1000	22
4	[100]:[0.1]	18	24 h	-	-

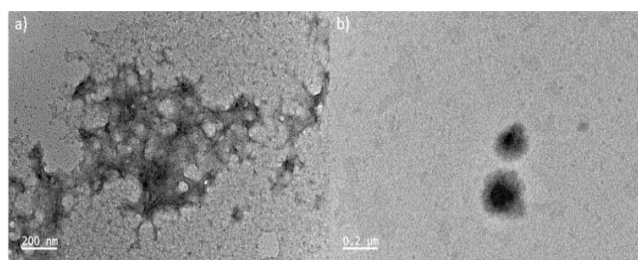


Fig. 3 TEM images of hydrogels prepared by type II photoinitiated polymerisation using [100]:[0.3] 24h (a), and [100]:[0.6] 6h (b) monomer to BP mole ratio

^a Determined by DLS measurements, ^b determined gravimetrically.

RhB to BP mole ratio was deliberately kept in excess (1:6), since RhB has four hydrogens, at the alpha position to the tertiary amine groups, that have the potential for radical generation to start polymerisation. When monomer concentration is set to 50 mg/ml and polymerisation is carried out for 24 h, the reaction yield is the highest; however, a coagulum was obtained, which might be attributed to the high monomer concentration in the feed (Table 1, entry 1). However, the hydrogel obtained is highly fluorescent and can swell in the aqueous environment, proving the success of the developed synthetic strategy (Fig. 2).

The following set of reactions were carried out to synthesise nano-size and coagulum-free hydrogels. For this purpose, reactions were carried out in more dilute conditions (18 mg/ml) to prevent particle aggregation. When the monomer concentration was decreased to 18 mg/ml and [Monomer]:[BP] mole ratio is set to [100]:[0.6], the reaction yield was 41% even after 6 h. Compared to the yields of reactions at the same monomer concentration, the relatively high yield of reaction might be attributed to the use of bimolecular initiator system in higher amount. At this stage, diffusion light scattering (DLS) results reveal the presence of uniform and discrete particles with 197 nm in size (Fig. S1). TEM images (Fig. 4b) showed similar characteristics, round shaped particles with a size of 192 ± 18 nm. As the [Monomer]:[BP] mole ratio is set to [100]:[0.3], the obtained particles were polydisperse, having diameters ranging from 200 nm to 1000 nm, and the reaction yield decreased to 22% even after 24 h irradiation. The increase in particle size might be attributed to the longer irradiation times and the lower reaction yield might be due to the decreased amount of initiator. TEM images (Fig. 3a) revealed sponge-like structures of various sizes and shapes, making it difficult to conduct accurate DLS measurements (Fig. S2). When the [Monomer]:[BP] mole ratio is further decreased to [100]:[0.1], any isolable product can be obtained after 24 h of reaction. It is important to note that, the colour of the obtained hydrogel is different than that of RhB (pink vs. orange), which might be attributed to the relatively long irradiation times that causes a partial photodegradation of RhB embedded in the polymer network. A control experiment was also carried out in the absence of RhB and BP, and no product was obtained after 24 h of irradiation, proving that the photoinduced polymerisation

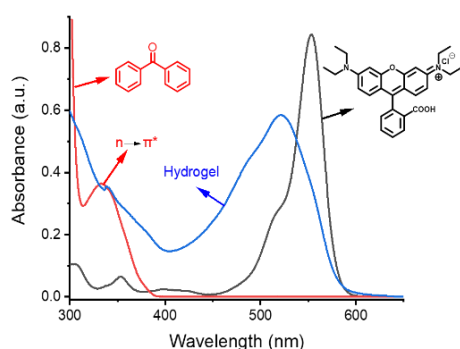


Fig. 4 UV-Vis spectra of BP, RhB, and the fluorescent hydrogel prepared by type II photoinitiated polymerisation.

pathways other than type II photoinitiated polymerisation are negligible, if not impossible.

Fig. 4 shows the UV-Vis spectra of the BP, RhB, and the fluorescent hydrogels recorded in ethanol. As can be seen, BP has a local absorption maximum at around 340 nm, which is responsible for the excitation of non-bonding electrons of the carbonyl oxygen, resulting in $n \rightarrow \pi^*$ transition.¹⁶ BP is deliberately selected as the photosensitiser molecule in the photopolymerisation reactions at 365 nm, since its wavelength of UV absorption responsible for $n \rightarrow \pi^*$ transition does not overlap the UV spectrum of RhB.

Fig. 5 shows the fluorescence excitation and emission spectra of the hydrogels prepared by using different initiator concentrations. When excited at 535 nm, both hydrogels exhibit a broad emission spectrum centred at around 600 nm, characteristic of RhB. When the monomer concentration in the feed is decreased by half, a slight decrease in the intensity of the emission spectrum is observed, indicating the presence of a RhB in both polymers prepared using [100]:[0.6] and [100]:[0.3] monomer to BP mole ratios, albeit to a lesser extent in the latter.

The presence of RhB groups in the nanogels, prepared by different initiator concentrations, is further supported by fluorescence microscopy as presented in Fig. 6. As can be seen, coagulum-free separate nanogel regions are observed for each

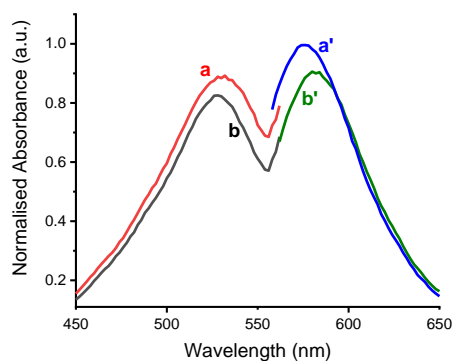


Fig. 5 Normalized excitation (a, b) and emission (a', b') fluorescence spectra of the hydrogels prepared by type II photoinitiated polymerisation using [100]:[0.6] (a), and [100]:[0.3] (b) monomer to BP mole ratio.

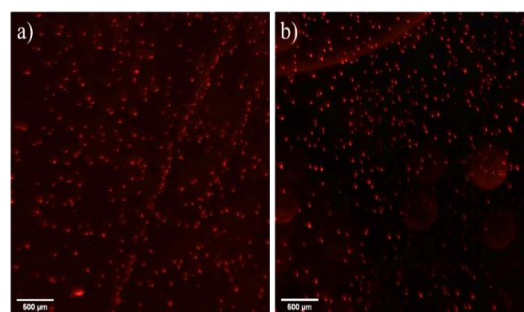


Fig. 6 Fluorescence microscopy images of hydrogels prepared by type II photoinitiated polymerisation using [100]:[0.6] (a), and [100]:[0.3] (b) monomer to BP mole ratio.

monomer concentration. Even though the resolution of the light microscope cannot be smaller than the half of the wavelength of the visible light (400 nm - 700 nm), it is still possible to observe the coagulum-free and discrete fluorescent particles in the sub-micron range. Under the same amount of irradiation intensity, particles prepared using higher initiator concentration possess higher fluorescence, confirming the presence of type II photoinitiation during polymerisation. This is also approved by the fluorescence spectrum obtained. In addition, each region occupied by hydrogels exhibits fluorescence with similar intensities, indicating the uniform distribution of the RhB during the polymerisation.

The presence of the RhB in the hydrogels is further supported by Fourier-transform infrared spectroscopy (FTIR). Fig. 7 shows the FTIR spectra of the fluorescent hydrogels prepared at different initiator concentrations. For comparative purposes, another hydrogel with identical monomer composition was also synthesised by replacing RhB and BP with 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (TTMA). Unsurprisingly, it was difficult to identify the presence of the RhB moieties from the FTIR spectrum (only 1-2% percent used in the feed) and all polymers exhibit peaks characteristic for polyacrylamide in their spectra (a broad peak centred at 3400 cm^{-1} , and sharp peaks at 1650 cm^{-1} , 2900 cm^{-1} are assigned for NH_2 , C=O, and C-H stretches, respectively). However, it was still possible to observe an increase in the intensity of the broad peak centred at around 3450 cm^{-1} ,

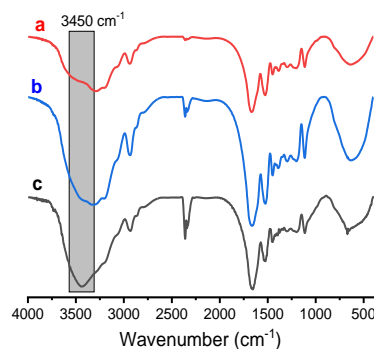


Fig. 7 FTIR spectra of the non-fluorescent hydrogel (a), and fluorescent hydrogels prepared by type II photoinitiated polymerisation using [100]:[0.3] (b), and [100]:[0.6] (c), monomer to BP mole ratios.

which is assigned to the O-H stretching vibration band arising from the carboxylic acid of RhB. As the RhB ratio is increased in the reactant composition, the intensity of the signal also increased (Fig. 8c), suggesting the successful incorporation of RhB into the polymer network.

In summary, a simple and cost-efficient method is developed for the synthesis of fluorescent hydrogels, using RhB as hydrogen donor in type II photoinitiation system. This new method yields highly fluorescent and uniform nanogels in a size range (from 200 nm to 1000 nm) attractive for many applications. The flexibility of irradiation wavelength for polymerization makes type II photoinitiators ideal candidates for the polymerisation of various vinyl monomers in the presence of photosensitive or thermosensitive compounds. In addition, by simply replacing RhB with other hydrogen donors, several other functionalities can be incorporated into the hydrogels in one step. Further studies in this line are now in progress.

All the authors acknowledge financial supports from Interface for the Living (IPV) - Sorbonne Université and Emergences Sorbonne Université.

There are no conflicts to declare.

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