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# **Photochemical & Photobiological Sciences**

# COMMUNICATION

## Photo-controlled growth of polymeric submicron-sized particles

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A tripodal coumarin derivative shows complex photoreactivity, changing from intra- to intermolecular photodimerization with increasing concentration. At high concentration, the compound undergoes efficient photopolymerization, resulting in the formation of polymeric submicron-sized particles. The size of these particles can be precisely increased through photoirradiation, without affecting their polydispersity.

Polymeric particles (PPs) have gathered much attention from the scientific community due to their important role in a wide spectrum of areas, including electronics and photonics, sensors, or medicine and biotechnology.<sup>1-6</sup> A recurring topic in PP research is the optimization of preparation methods that greatly influence their final properties. In particular, size and morphology of PPs are crucial factors that affect their applicability, since they determine key properties such as viscosity, surface area and packing density.<sup>7-8</sup> When PPs are used as drug delivery carriers, size defines their biological effect, cellular internalization mechanism and the in vivo fate of the drug.<sup>9-11</sup> Thus, methods that allow the preparation of particles with specific sizes and structures with high reproducibility and in relatively high volumes are a major objective of material science research. Most PP preparation methods involve the precipitation of preexisting polymers or the polymerization of monomers in nanosized droplets, which require the addition of stabilizers and/or additional reagents.

While this does not pose significant purification problems at laboratory scale, considerable challenges emerge when large production volumes are needed. From this perspective, we envision that *in situ* photopolymerization can lead to the formation of polymeric particles from homogenous solutions,

exempt of elaborate purification steps and without additional reagents. Using a tripodal motif bearing photodimerizable moieties (Scheme 1), the preparation of polymeric particles of precise dimensions and narrow size distribution can be achieved. Coumarins, which are naturally occurring chromophores with tunable photophysical and photochemical properties, are known to undergo photodimerization and make for ideal candidates for the functionalization of the tripodal molecules.<sup>12-14</sup>

The synthesis of tripodal precursors bearing a coumarin chromophore (3) is readily achieved from the derivatization of coumarin-3-carboxylic acid (1) with thionyl chloride, followed by condensation with tris(2-aminoethyl)amine, tren, in the presence of potassium carbonate (Scheme 2). Compound 3 was obtained in good overall yield (85%) and at high purity after a simple purification process that does not require column chromatography (see Supplementary material). Compound 3 is moderately soluble in acetonitrile and dimethylsulfoxide, and highly soluble in chloroform. The absorption spectrum of a freshly prepared solution of 3 exhibits two bands without vibrational structure in the UV region at approximately 300 and 350 nm, corresponding to the typical  $\pi$ - $\pi$ \* transitions associated to the charge transfer from the benzenic cycle to the pyranone moiety of coumarins (Figure 1).<sup>15</sup> Due to the presence of an electron-withdrawing amide moiety group at position 3, the absorption maxima of compound **3** are shifted bathochromically (ca. 30-40 nm) in comparison with unsubstituted coumarin.<sup>16</sup> In addition, the calculated molar absorptivity value ε of ca. 38900 M<sup>-1</sup> cm<sup>-1</sup> is approximately three times higher than that of native coumarin (ca. 9000-12500  $\mbox{M}^{\mbox{-}1}$ cm<sup>-1</sup>, depending on solvent polarity), due to the presence of three independent chromophores in the tripodal molecule.<sup>15,16</sup>

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Scheme 1 - Representation for light-controlled dendrimeric growth of nanoparticles from photopolymerizable tripodal molecules



Scheme 2 - Synthetic Pathway for the Preparation of Coumarin Derivative 3

Upon irradiation at the absorption maxima, there is a clear decrease of the absorption of both bands (Figure 1), compatible with a loss of conjugation length of the coumarin chromophores. In this wavelength range, only a very small increase below 250 nm is noticed. The spectral transformations observed are consistent with the photodimerization reaction of the coumarin moiety<sup>17</sup>. This hypothesis is further corroborated by <sup>1</sup>H NMR spectroscopy, since the spectra obtained after irradiation show the characteristic peaks of the cyclobutane moieties formed upon dimerization of coumarins (ESI, Figure S1 and S3).

Since the photodimerization can occur from both intramolecular and intermolecular processes, and only intermolecular dimerization can give rise to the desired photopolymerization, elucidation of the photochemical mechanism was required. Because photochemical bimolecular processes are concentration sensitive,<sup>18</sup> irradiation experiments over a broad concentration range were conducted and the respective photochemical quantum yields calculated (Table 1). The results show a clear correlation between the concentration of 3 and the photochemical quantum yield for its disappearance ( $\Phi_R$ ), indicating that there is a significant contribution of intermolecular photodimerization to the observed photoreactivity.

Table 1 -Determined photochemical quantum yield values,  $\Phi_{\text{R}}$  for the disappearance of 3 upon irradiation and calculated values for unimolecular.  $\Phi_{\rm d}$ , and bimolecular.  $\Phi_{\rm b}$ reactions at different concentration of 3 in chloroform.

concentration (10 <sup>-5</sup> M)	$\Phi_{R^{a}}$ (%)	$\Phi_{d^{b}}$ (%)	Φ <sub>D</sub> c (%)
0.56	1.02	0.97	0.05
1.15	1.09	0.95	0.14
3.67	1.21	0.85	0.36
11.2	1.52	0.71	0.81
50.2	1.85	0.34	1.51
473.2	1.99	0.05	1.94

a) determined by UV-Vis and NMR spectroscopy at conversion rates <5% b) calculated from equation (2)

c) calculated from equation (3)

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fitting parameters:  $\frac{k_d}{k_{NR}}$ =0.01,  $\frac{k_D}{k_a}$ =0.02,  $\frac{k_d}{k_D}$ =0.37 and  $k_A$ = 4630 M<sup>-1</sup>



Figure 1 - Spectral modifications of 2.6 x 10<sup>-5</sup> M **3** in chloroform upon irradiation at 330 nm. Under the experimental setup, the photostationary state was reached after 60 min.

This phenomenon can be rationalized if a simplified model for the photodimerization reaction, where no photodegradation occurs, is considered, as depicted in Scheme 3. In this model, M and  $M^*$ represent compound 3 in the ground and excited states, respectively, (MM\*) the excimer formed by an excited and a ground state molecule of **3**, *D* the (ground state) dimer of two molecules of **3** and *d* represents compound **3** with two intramolecularly dimerized coumarin moieties. The quantum yields for the intramolecular and intermolecular dimerization processes,  $\Phi_D$  and  $\Phi_d$ , respectively, are given by equations (1) and (2):

$$\Phi_D = \frac{k_D k_A[M]}{k_d + k_{NR} + k_D k_A[M] + k_q k_A[M]}$$
(1)  
$$\Phi_d = \frac{k_d}{k_d + k_{NR} + k_{NR} + k_{NR}[M]}$$
(2)

$$d = \frac{1}{k_d + k_{NR} + k_A[M]}$$

where  $k_{d}$  and  $k_{D}$  are the rate constants for unimolecular and bimolecular dimerizations, respectively,  $k_A$  the (MM\*) excimer formation constant,  $k_q$  the excimer self-quenching rate constant and  $k_{\rm NR}$  is the rate constant for the non-reactive decay of compound 3.

The quantum yield for the observed reaction, i.e., disappearance of **3**,  $\Phi_{R}$ , is given by the sum of both processes:  $\Phi_R = \Phi_D + \Phi_d$ (3)

Thus, for dilute solutions, the observed photochemical reaction should arise only from an intramolecular process,

$$\Phi_R^0 = \Phi_D^0 + \Phi_d^0 = \frac{k_d}{k_d + k_{NR}} ([M] \rightarrow 0)$$
(4)  
and for concentrated solutions, the opposite is verified.

$$\Phi_R^{\infty} = \Phi_D^{\infty} + \Phi_d^{\infty} = \frac{k_D}{k_D + k_q} \left( [M] \to \infty \right)$$
(5)

$$M+M \stackrel{k_{\rm NR}}{\longrightarrow} M^* + M \stackrel{k_{\rm A}}{\longrightarrow} (MM^*) \stackrel{k_{\rm D}}{\longrightarrow} D \stackrel{M+M}{\longrightarrow} M + M$$

Scheme 3 - Kinetic model for photodimerization reaction occurring in tripod 3

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Using equations 1-3, it is possible to fit the experimental values of  $\Phi_R$  with great accuracy and calculate the contribution of intra- and intermolecular dimerization at each concentration value (Figure 2, Table 1). The fitting was achieved using the kinetic relationships  $\frac{k_d}{k_{NR}}$ =0.01,  $\frac{k_D}{k_q}$ =0.02,  $\frac{k_d}{k_D}$ =0.37 and  $k_A$ = 4630 M<sup>-</sup> <sup>1</sup>. Figure 2 evidences that for concentrations of **3** below 10<sup>-4</sup> M, the efficiency of intramolecular dimerization is higher than that of intermolecular process, and photopolymerization cannot occur below this concentration. In addition, to achieve a significant polymerization degree,  $\Phi_D$  must be higher than  $\Phi_d$ at a high conversion range, otherwise, an inversion in the process relative efficiency can occur during irradiation. Thus, solutions of 3 with concentrations above 1 mM were irradiated, as this concentration ensures that  $\Phi_D > \Phi_d$  up to 90% conversion of 3. It was observed that the irradiated solutions turned turbid due to the formation of an insoluble precipitate. Analysis of the suspensions by atomic force microscopy (AFM) revealed the presence of spherical nanoparticles with narrow size distributions (Figure 3a). These results were corroborated by Dynamic Light Scattering (DLS), with the particles exhibiting a mean particle diameter of 100 nm and polydispersity index (PDI) of 0.2. It is proposed that these results arise from the photopolymerization of 3 upon irradiation, which leads to the precipitation of insoluble oligomers. Further irradiation of the obtained suspensions resulted in a significant increase in particle size. For instance, after 400 minutes of continuous irradiation, particles with a mean diameter size of ca. 400 nm were observed (Figure 3b). It is worth noting that upon this 4fold increase in particle diameter, the PDI value of the suspension did not vary significantly (ca. 0.23), which suggests that the increase in size results from the growth of the initial particles, rather than their aggregation.<sup>19</sup> Therefore, it is growth mechanism proposed that the involves photodimerization reactions occurring at the surface of the polymeric particles between their coumarin moieties and free molecules or small oligomers of 3.



Figure 2 - Observed photodimerization quantum yield ( $\Phi_R$ , square dots) dependency with concentration of 3 and theoretical fitting (eqns. 1-3) of overall (solid black line), intermolecular (dott $\underline{k}_d$  red lin $\underline{k}_p$ ) and intramolecular (dashed blue line) quantum yields. Fitting parameters:  $\overline{k_{NR}}$ =0.01,  $\overline{k_a}$ =0.03,  $\overline{k_b}$ =0.37 and  $k_A$ = 4630 M<sup>-1</sup>



Figure 3 - Atomic force microscopy images obtained from the irradiation of a 10 mM solution of 3 in chloroform after 10 (a) and 400 (b) minutes. Scale bar is 1  $\mu$ m. Insets show size distribution determined by DLS.

The irradiation-induced growth rate was investigated with DLS spectroscopy and it was evidenced that it was virtually linearly dependent with the irradiation time (Figure 4). The same measurements were conducted for solutions of **3** at different concentrations and it is evidenced that the growth rate of the polymeric particles is significantly affected by the concentration of **3**.



Figure 4 - Nanoparticle size dependence with irradiation time and concentration of 3 in chloroform.

In conclusion, the results obtained for tripodal coumarin **3** derivative show that it is possible to obtain particles with the desired size in the submicron regime by adjusting the duration of exposure of photopolymerizable compounds to UV light, under determined irradiated conditions. By tuning the photoreaction mechanism through concentration adjustment, efficient photopolymerization was achieved and resulted in the formation of polymeric nanoparticles. The size of these particles could be accurately controlled by adjusting irradiation time and monomer concentration, without affecting the polydispersivity. The present work evidences the potential of photoresponsive systems for the preparation of polymeric nano- and micromaterials with controlled size and morphology using a simple methodology exempt from additional organic reactants and purification steps.

## **Conflicts of interest**

There are no conflicts to declare.

## Acknowledgements

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A tripodal coumarin derivative is photopolymerized into polymeric nanoparticles with 100 nm. The size of the polymeric particles can be accurately tuned within the submicron range with irradiation time.