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Additional Information

Photobehavior of the antipsychotic drug cyamemazine in a **supramolecular gel** protective environment

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ABSTRACT

In this work, a molecular hydrogel made of gelator (*S*)-4-((3-methyl-1-(nonylamino)-1-oxobutan-2-yl)amino)-4-oxobutanoic acid (**SVN**) has been employed as soft container to modify the photochemical and photophysical behavior of the antipsychotic drug cyamemazine (**CMZ**). The interaction of **CMZ** with the gel network has been evidenced by fluorescence spectroscopy through a hypsochromic shift of the emission band (from $\lambda_{\text{max}} = 521$ nm in solution to $\lambda_{\text{max}} = 511$ nm in the gel) and an increase of the fluorescence lifetime (5.6 ns in PBS vs. 7.2 ns in the gel). In the laser flash photolysis experiments on **CMZ/ SVN** systems, the **CMZ** triplet excited state $^3\text{CMZ}^*$, monitored at $\lambda = 320$ nm, has been more efficiently generated and became much longer-lived than in solution (2.7 μs vs. 0.7 μs); besides, photochemical ionization leading to the radical cation $\text{CMZ}^{\cdot+}$ was disfavored. In the steady-state experiments, photooxidation of **CMZ** to afford the *N,S*-dioxide derivative **CMZ-SONO** has been retarded in the gel, which provides a more lipophilic and constrained microenvironment. Both the photophysical properties and the photoreactivity are in agreement with **CMZ** located in a less polar domain when entrapped in the supramolecular gel, as result of the interaction of the drug with the fibers of the supramolecular **SVN** gel.

1. Introduction

Cyamemazine (**CMZ**, Chart 1), 10-[3-(dimethylamino)-2-methylpropyl]phenothiazine-2-carbonitrile, is a neuroleptic drug that contains the phenothiazine chromophore [1,2]. Concerning its photophysical properties, the **CMZ** fluorescence spectrum displays a maximum at $\lambda = 530$ nm, with a fluorescence quantum yield (ϕ_{F}) of 0.11 in PBS (phosphate buffered saline solution, pH = 7.4); the absorption spectrum obtained upon laser flash photolysis (LFP) exhibits contributions from two transient species: the triplet excited state ($^3\text{CMZ}^*$) and the radical cation ($\text{CMZ}^{\cdot+}$). Both species overlap in the 400-550 nm region; at shorter wavelengths, $^3\text{CMZ}^*$ peaks at 320 nm, while $\text{CMZ}^{\cdot+}$ does at 290 nm [3,4].

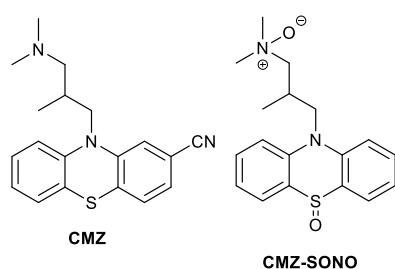


Chart 1. Chemical structures of the investigated drug and its photoproduct

Upon UVA steady-state irradiation, **CMZ** photoionizes to afford $\text{CMZ}^{\cdot+}$, which is trapped by oxygen to afford the corresponding *N,S*-dioxide derivative (**CMZ-SONO**, also shown in Chart 1) [5]. This photoproduct has been claimed to play a major role in the phototoxicity of **CMZ**, due to its ability to produce singlet oxygen and thus acting as Type-II photosensitizer. Our group has recently examined the photobehavior of **CMZ** within different microenvironments. The results show that, in sodium dodecyl sulphate micelles and in α_1 -acid glycoproteins, the local hydrophobicity prevents **CMZ** photoionization, resulting in a retarded photooxidation [4].

Supramolecular gels, unlike polymer-based gels, are built from low molecular weight units that self-assemble forming simple spanning fibrillary networks [6-8]. These soft materials have been extensively studied during the last two decades, in connection with their unique properties (i.e. stimuli responsiveness) [9,10]. Besides, they have also been envisaged for applications in different fields including controlled release, optoelectronic soft materials, catalysis, tissue engineering, etc. [11-13]

Specifically, **supramolecular gels** made from gelators with catalytic or reactive moieties have been used as soft containers for chemical reactions [14]. For example, **physical gels** containing *p*-nitrophenyl carbamate moieties undergo reaction with amines to yield ureas [15]. Gels formed from gelators containing histidine or proline units have shown catalytic activity in solvolysis or aldol reactions, respectively [16,17].

Much less exploited has been the use of **supramolecular gels** as media for the study of photophysical and photochemical processes [18-22]. For example, a molecular organogel from a binary gelator containing 2-anthracenecarboxylic acid undergoes stereoselective photodimerization, which is heavily influenced by gelation [23]. Likewise, the lifetime of a photomerocyanine, formed by photoactivated spiroyrans ring opening, is dramatically enhanced in the presence of an organogel [24]. Another illustration is a bile acid derived molecular hydrogel that has been found to modify the stereoisomer distribution of the acenaphthylene photodimerization products [25]. In the two last mentioned cases, interaction of the involved species with the self-assembled chiral fibers is claimed to rationalize the observed effects. In a different approach, a photooxidation catalyst (riboflavin tetraacetate), entrapped in an organogel has been used to achieve efficient oxidation of benzyl alcohol [26].

Supramolecular gels can be envisaged as suitable nanoreactors. Thus, the parent low-molecular weight gelators can be easily synthesized from commercially available building blocks, and the presence of amide groups produces, under appropriate conditions, a monodimensional arrangement of fibers leading to the gel. In the case of L-valine, the hydrogel based on gelator (*S*)-4-((3-methyl-1-(nonylamino)-1-oxobutan-2-yl)amino)-4-oxobutanoic acid (**SVN**, Chart 2) fulfils the appropriate optical requirements to act as host of photochemical reactions [27].

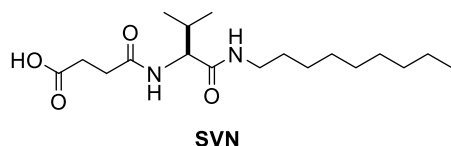


Chart 2. Chemical structure of **SVN** gelator

With this background, we have selected **CMZ** as photoactive probe and a gel based on **SVN** gelator to gain insight into the influence of **this hydrogel** on photophysical properties and on photooxidation processes.

2. Results and Discussion

2.1. Synthesis of the Materials

Gelator **SVN** was obtained in a four-step synthesis, starting from *N*-Cbz-protected valine [20,26]. The corresponding gel was obtained by heating/cooling treatment of a PBS solution of the appropriate concentration of **SVN** (see experimental section for details). **Gel-like appearance was confirmed by the vial inversion test.**

2.2. Photophysical Measurements

First, the singlet excited state properties of **CMZ** were investigated in phosphate buffered saline (PBS) solution and in a **SVN/PBS** gel. Fig. 1A shows the normalized emission spectra of **CMZ** at $\lambda_{exc} = 330$ nm in both media. Interestingly, a small but significant hypsochromic shift from $\lambda_{max} = 521$ nm (PBS) to $\lambda_{max} = 511$ nm (gel) was observed. Concerning the time-resolved emission measurements (Fig. 1B), the fluorescence lifetime (τ_F) was longer in the gel media than in solution (7.2 ns vs. 5.6 ns). These findings point to a more lipophilic and constrained microenvironment in the gel.

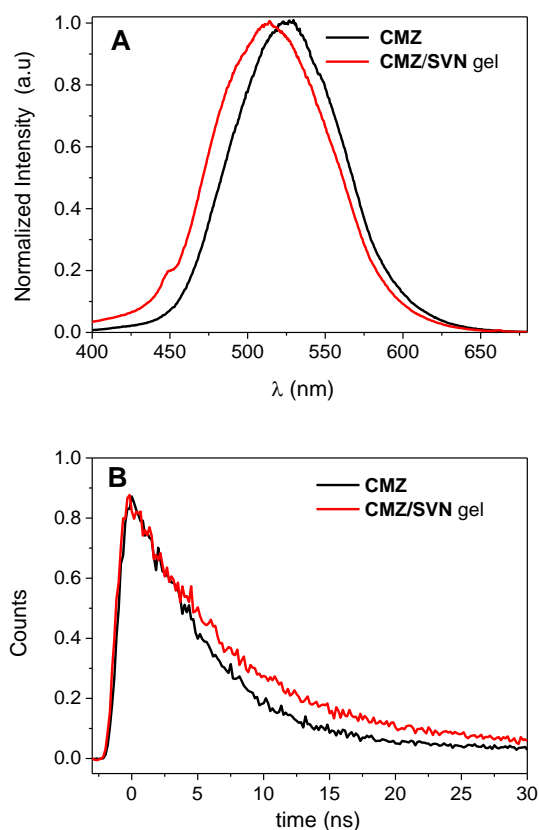


Fig. 1. **A:** Normalized emission spectra of **CMZ** at $\lambda_{exc} = 330$ nm. **B:** Fluorescence decays of **CMZ** at $\lambda_{exc} = 340$ nm. Conditions: [**SVN**] = 6.3×10^{-3} M [**CMZ**] = 2×10^{-5} M, PBS, air.

Then, laser flash photolysis experiments were performed on the same systems. Thus, while the contribution of **CMZ⁺⁺** ($\lambda = 290$ nm) predominated in the transient absorption spectra in PBS, it was less prominent in the **SVN** gel, where the band due to **³CMZ*** ($\lambda = 320$ nm) was clearly observed (Fig. 2A). A considerable triplet lifetime increase was also observed upon going from the solution to the gel (0.7 μ s vs. 2.7 μ s, Fig. 2B).

2.3. Steady-state irradiations

The drug was monochromatically irradiated at $\lambda = 355$ nm in PBS and in **SVN/PBS**, under air. The progress of the reaction was followed by monitoring the variations in the fluorescence spectra (at $\lambda_{exc} = 330$ nm) with the irradiation time (Figs. 3A and 3B). This allowed detecting formation of photooxidation product **CMZ-SONO**, by following the growth of its characteristic emission band in the 380-459 nm region.

The kinetics of the process in the two media can be compared by plotting the relative intensities at $\lambda = 405$ nm vs. time (Fig. 3C). Clearly, photooxidation resulted to be markedly retarded in gel media. Therefore, **CMZ** protection against photooxidation to **CMZ-SONO** was higher in **SVN** gel media, where triplet is much longer-lived than in PBS solution. This does not support involvement of **³CMZ*** as the key intermediate in the reaction mechanism, and is in good agreement with **CMZ⁺⁺** trapping by oxygen leading to **CMZ-SONO**. [4]

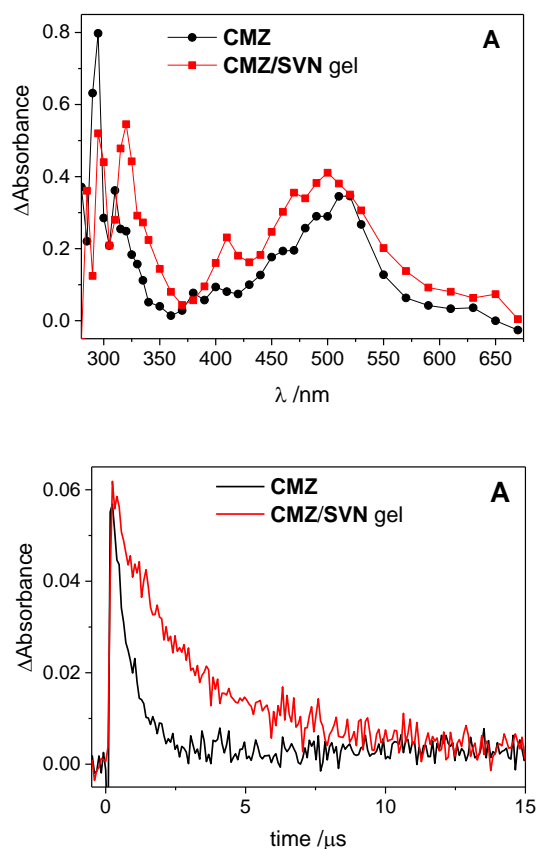


Fig. 2. Laser flash photolysis of CMZ. **A:** Transient absorption spectra obtained 1.4 μ s after the laser pulse. **B:** $^3\text{CMZ}^*$ decays monitored at $\lambda = 320$ nm. Conditions: $\lambda_{\text{exc}} = 355$ nm, $[\text{SVN}] = 6.3 \times 10^{-3}$ M, $[\text{CMZ}] = 2 \times 10^{-5}$ M, PBS, air.

3. Conclusions

The interaction of CMZ with SVN gel can be monitored by fluorescence spectroscopy. Thus, in steady-state experiments a 10 nm hypsochromic shift of the emission band is observed, while approximately 30% increase of the fluorescence lifetime is noticed.

Transient absorption spectroscopy also allows detecting significant changes linked to the gelation process. In particular, the triplet excited state results to be more efficiently generated and becomes much longer-lived than in PBS solution. In addition, CMZ photochemical ionization leading to the radical cation $\text{CMZ}^{+\cdot}$ is reduced.

Photolysis of CMZ results in oxidation to CMZ-SONO. The process can be conveniently followed by fluorescence spectroscopy, monitoring the growth of the typical emission of CMZ-SONO centered at *ca.* 420 nm.

Overall, the abovementioned CMZ photophysical properties can be satisfactorily correlated with its photoreactivity. Photooxidation is retarded in the supramolecular gel. One possible explanation is that CMZ resides in a less polar domain, and thus formation of the radical cation is prevented. The same trend has been observed in other biomimetic media; the extent of photoprotection in the gel is higher than within cyclodextrins but lower than in the protein cavities. [4]

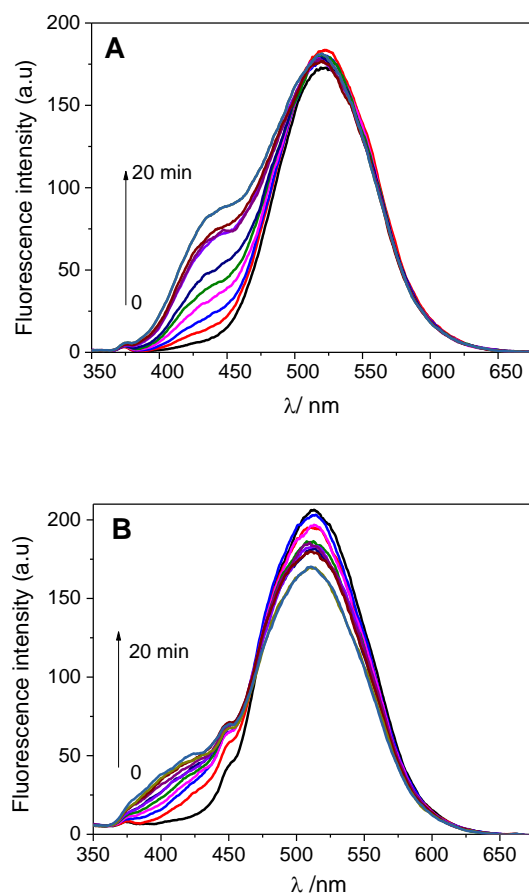


Fig. 3. Photooxidation of CMZ ($\lambda_{\text{irr}} = 355$ nm) monitored by fluorescence spectroscopy. **A:** PBS solution; **B:** SVN gel in PBS; **C:** comparison of the kinetics of CMZ photooxidation from the fluorescence measurements. Conditions: $\lambda_{\text{exc}} = 330$ nm, $[\text{SVN}] = 6.3 \times 10^{-3}$ M, $[\text{CMZ}] = 2 \times 10^{-5}$ M, air.

4. Experimental Section

4.1. General

Commercial solvents (spectroscopic and HPLC grade) were used as provided. Buffered phosphate saline (PBS, 0.01M, pH =7.4) solutions were prepared from commercial tablets. Racemic CMZ tartrate was purchased from Aventis and kept at 4° in the absence of light. Free CMZ was obtained by extraction of the CMZ tartrate with 1M NaOH/ CH_2Cl_2 . After drying with Na_2SO_4 , filtration and evaporation *in vacuo*, racemic CMZ was obtained [4]. Photophysical measurements have been performed three times

using two **CMZ** or **CMZ/SVN** samples each time, to check the reproducibility of the results. In all cases the results were the same.

4.2. Synthesis of gelator SVN and macroscopic The SVN hydrogel preparation

The synthesis of SVN gelator was performed according to the procedure described in ref. 27. For the gel preparation, a mixture of 4.3 mg of SVN in 2 mL of PBS were introduced into a cylindrical screw-capped glass vial (8 mL, diameter = 1.5 cm) and heated until completed solution of the gelator. Then it was placed in a 1 cm × 1 cm quartz cell and cooled to 25 °C. Formation of the gel was proven by means of the vial inversion test.

4.3. Fluorescence and Laser Flash Photolysis Experiments

The equipments employed for recording the emission spectra, for determining the fluorescence lifetimes and for transient absorption spectroscopic measurements have been described elsewhere [28,29]. Experiments were performed at 22 °C; concentration of **CMZ** was 2×10^{-5} M and concentration of **SVN** was 6.3×10^{-3} M.

4.4. Steady-state Irradiations

Solutions of **CMZ** in PBS or in **SVN/PBS** gel were placed into 1 cm × 1 cm quartz cells and exposed to monochromatic light ($\lambda_{exc} = 355$ nm) for different times. The progress of the reaction was monitored by following the variations in the fluorescence intensity at $\lambda_{exc} = 330$ nm. Concentration of **CMZ** was 2×10^{-5} M and concentration of **SVN** was 6.3×10^{-3} M.

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