



Drug Loading Study of Chlorambucil-TEMPOL Adduct in R_f-PEG Hydrogel via Electron Spin Induced ¹⁹F Spin Lattice Relaxation NMR and EPR Spectroscopy



Xiangli Liu¹, Anuja Prabhutendolkar¹, Errol Mathias¹, Yougang Mao¹, Yong Ba¹, and Julie A. Kornfield²

¹California State University Los Angeles: Department of Chemistry and Biochemistry; Los Angeles, CA 90032

²California Institute of Technology: Division of Chemistry and Chemical Engineering; Pasadena, CA 91125

ABSTRACT

6KC6(PEG 6K, C₆F₁₃CH₂OH), one of the two-phase R_f-PEG's (fluoroalkyl polyethylene glycols) that can form a sol-gel coexisting hydrogel was loaded with an hydrophobic drug to study its hydrophobic drug delivery mechanisms. Chlorambucil, a hydrophobic anti-cancer drug was selected for the study of drug delivery, and it was spin labeled by a nitroxide radical, TEMPOL (4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-N-oxyl) covalently. The Chlorambucil-TEMPOL(CT) Adduct loaded R_f-PEG (6KC6) hydrogel was prepared by a method of probe sonication. Drug loading is the first step for drug delivery study and the loading sites have been proven to be inside the cores of the R_f-PEG micelles by EPR method in our previous research. To determine the maximum loading concentration, EPR and ¹⁹F T₁ relaxation NMR were carried out for 6KC6 hydrogel samples loaded with different CT Adduct concentrations. Our experimental data shows that the ¹⁹F T₁ relaxation time decreases with the increase in the drug loading level. The maximum or saturated loading level was determined by the level-off of the ¹⁹F T₁ relaxation time. The drug-loading study by the ¹⁹F T₁ method complements that by the EPR method. Saturated drug loading level depends on hydrophobicity of drug, size of R_f-PEGs and concentration of hydrogel. 2% CT /6KC6 in 10% hydrogel was estimated to be the maximum loading concentration probed by EPR spectra and NMR ¹⁹F spin lattice relaxation times.

INTRODUCTION

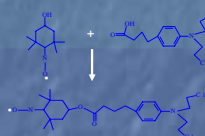
Fluoroalkyl Polyethylene Glycol or R_f-PEG is functionally modified polyethylene glycol (PEG) with two hydrophobic fluoroalkyl (R_f) terminal groups. R_f-PEG's with certain middle block and end lengths exhibits interesting properties of sol-gel phase coexistence and surface erosion which renders this material a rare candidate as hydrophobic drug-delivery depot to obtain controlled and sustained drug release rate. We have synthesized an electron spin-labeled hydrophobic drug by covalently attaching TEMPOL (4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-N-oxyl) to Chlorambucil. Chlorambucil with a Brand name of Leukeran, belonging to the family of alkylating agents, is an anticancer drug mainly used to treat cancer of blood and lymph system. TEMPOL is a stable free radical with an unpaired electron primarily located in the π-orbital of the nitroxide group. The Chlorambucil-TEMPOL Adduct loaded R_f-PEG (6KC6) hydrogel was prepared by method of probe sonication. Drug loading is the first step for drug delivery study and the loading sites have been proven to be inside the cores of the R_f-PEG micelles by EPR method. Nuclear spin lattice relaxation is generally dominated by magnetic dipole-dipole interaction of a nucleus with its surrounding, and the relaxation rate depends on the magnitude of the magnetic moments of the interacting spins. Since the electron magnetic moment is much larger than nuclear magnetic moment, the ¹⁹F T₁ rate in this study is dominated by the electron-nuclear dipole-dipole interaction. Therefore, the ¹⁹F spin lattice relaxation is significantly affected by the number of interacting electron spins. Our experimental data show that the ¹⁹F T₁ relaxation time decreases with the increase in the drug loading level. The maximum or saturated loading level was determined by the level-off of the ¹⁹F T₁ relaxation time. The drug-loading study by the ¹⁹F T₁ method complements that by the EPR method.

EXPERIMENTAL

1. Materials

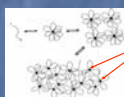
> R_f-PEG of 6KC6: C₆F₁₃-CH₂-CH₂-O-IPDU-o-(CH₂CH₂O)_n-IPDU-O-CH₂CH₂-F₁₃C₆ was synthesized by method in reference #3

> Spin-labeled drug, Chlorambucil TEMPOL(CT Adduct):



> CT Loaded hydrogel of 6KC6: Prepared by method of probe sonication

Figure 1: Micelle networking in the 2 phase R_f-PEG's



Loading sites of Hydrophobic drug

2. Instrumentation

- > NMR relaxation times were recorded on a 400 MHz Bruker Avance system with a 5mm QNP probe.
- > EPR spectra were recorded on a X-band Bruker-EMX EPR Spectrometer.

RESULTS

1. ¹⁹F T₁ Relaxation Times



Figure 2a: Typical ¹⁹F T₁ curves observed for the terminal -CF₂ of the R_f group

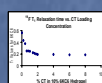


Figure 2b: Graph results for the T₁ relaxation times of the hydrogel loaded with various concentrations of CT

2. EPR Spectra for CT loaded 6KC6 Hydrogel in different concentrations

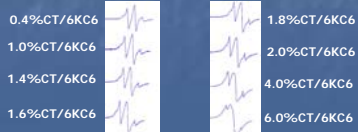


Figure 3: EPR spectra of the hydrogel with various concentrations of CT loading

DISCUSSION

NMR ¹⁹F T₁ relaxation times

¹⁹F T₁ NMR relaxation times were measured for 12 samples of the CT Adduct loaded 6KC6 hydrogel in different ratios of CT/6KC6 from 0.2% to 8%, and 1 blank 10%/6KC6 hydrogel sample. The blank sample has highest T₁ value, then T₁ decreases with CT ratio increases from 0.2% to 2%; T₁ time becomes constant when the loading ratio is over 2%. Therefore, the maximum loading level for 10% 6KC6 hydrogel is below 2% CT/6KC6 as seen in Figure 2b.

EPR Spectra

Line shape and line width are similar in the case of loading ratios below 2%CT/6KC6; Serious line broadening and line shape changes were observed in 4% and 6% due to precipitation outside cores of micelles in hydrogel. 2% CT/6KC6 was estimated to be the maximum loading level based on qualitative analysis of EPR spectra.

CONCLUSION

- T₁ relaxation NMR was used for the first time to determine drug loading characteristics of R_f-PEG hydrogels.
- The NMR and EPR studies were found to be complementary.
- This experiment proved that NMR can be used to quantitatively determine the maximum drug loading for a hydrogel.

FUTURE FOCUS

- Quantitative analysis and simulation of EPR spectra
- Study of different R_f-PEG's by T₁ relaxation NMR.
- Theoretical evaluation of drug loading by T₁ techniques.

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- Dr Gang Zhao

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