



# Study of the Delivery of the Hydrophobic Drugs by Fluoroalkyl Terminated Polyethylene Glycol Hydrogels using NMR and ESR Spectroscopy

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## ABSTRACT

In the pursuit of delivering hydrophobic drugs, Polyethylene Glycol (PEG) with terminally substituted fluoroalkyl (R<sub>f</sub>) groups were synthesized. The substitution can be either single or double ended. Also, the phase behavior of the R<sub>f</sub>-PEG's depends on the chain length of the PEG and R<sub>f</sub> groups thus forming single-phase (gel) or two-phase (sol-gel) hydrogel systems. It is known that these R<sub>f</sub>-PEG's have a very unique property of forming micelle-like structures with the hydrophobic (R<sub>f</sub>) ends forming the cores and the hydrophilic (PEG) groups protruding outwards. In order to investigate the delivery of hydrophobic drugs by R<sub>f</sub>-PEG's we have synthesized an electron spin-labeled derivative of a hydrophobic drug Chlorambucil with TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl). The unpaired electron in TEMPOL is primarily located in the pi-orbital of the nitrogen atom and gives rise to a hyperfine triplet in the ESR spectrum. The properties of the encapsulated drug molecules inside the cores of the R<sub>f</sub>-PEG's micelles are studied by the ESR spectra, and the <sup>19</sup>F-NMR T1 relaxation times influenced by the electron spins attached to the Chlorambucil molecules.

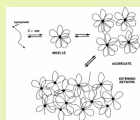


Fig1. Formation of micelles with the F groups at the core and linkage between different micelle groups in a Rf-PEG hydrogel system.

## INTRODUCTION

Fluoroalkyl-ended poly(ethylene glycol) (R<sub>f</sub>-PEG) hydrogels were designed with the biocompatibility and biodegradability in mind. A striking feature of this system is that the gel phase and the sol phase can co-exist. These properties predict the potential of the R<sub>f</sub>-PEG system to be developed as delivery depot to release hydrophobic drugs to the body with sustained and controlled release rates. Chlorambucil is a hydrophobic drug that is used to kill or control neoplastic cells; hence, used in chemotherapy to treat chronic lymphocytic leukemia, giant follicular lymphoma, and Hodgkin's disease. hemolytic anemia with cold agglutinins. The unpaired electron in TEMPOL is primarily located in a pi-orbital on the nitrogen atom. Interaction of the electron with the magnetic moment of <sup>14</sup>N-nucleus (99.73% natural abundance) which has a spin I = 1 gives rise to a hyperfine triplet spectrum with equal intensities. These hyperfine splittings vary with the orientation with the nitroxide group determined by the hyperfine splitting tensors. The solution ESR spectrum from the nitroxide molecules, which rotate randomly with correlation time < 10<sup>-9</sup> s, shows three sharp lines due to the average of the spectra from the entire orientations. The general lineshapes are affected by the rate and mode of the molecular motion and by the molecular orientation. Thus, the ESR of nitroxide labeled molecules represent sensitive probes for the study of molecular structure, dynamics, and interactions. Labeling chlorambucil with TEMPOL permits us to study the interaction of the hydrophobic drug with the R<sub>f</sub>-PEG polymers. The T1 relaxation times of the <sup>19</sup>F R<sub>f</sub> groups are affected by the proximity of the spin labeled drug and subsequently the peak shape in the ESR spectrum of the spin label changes due to interactions with the fluorine groups in the micelle. Further, we also studied the <sup>1</sup>H T1 relaxation times of the PEG backbone of the hydrogel. Along with the drug, the interaction of the parent spin label Tempol with the hydrogel was also studied.

## EXPERIMENTAL

1. Synthesis of 10KC<sub>8</sub> R<sub>f</sub>-PEG was obtained as per-mentioned in the reference #3.
2. Synthesis of Spin labeled drug  
To a stirred solution of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (0.172 g, 1 mmol) and chlorambucil (0.304 g, 1mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 ml) at 0°C under argon, DCC (0.206 g, 1 mmol) and DMAP (0.0307 g, 0.25 mmol) were added and reaction mixture was stirred for 12 h at room temperature. The solid materials formed were filtered off, and the filtrate was washed with 1 M HCl (1 ml) followed by saturated NaHCO<sub>3</sub> (2 ml) and brine (2 ml). The organic phase was dried over MgSO<sub>4</sub> and evaporated in vacuo. In order to characterize the structure of the molecule by using NMR spectroscopy, the nitroxyl free radical was reduced by using isoscorbic acid.

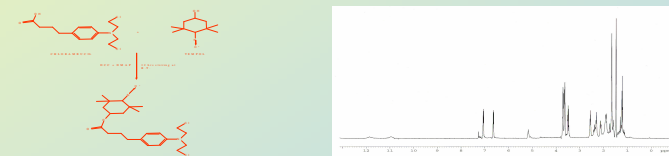


Fig.2. Schematic diagram for the synthesis of spin labeled chlorambucil and Fig.3. NMR spectrum of reduced product in CDCl<sub>3</sub> solvent.

3. Preparation of the hydrogel  
100mg 10KC<sub>8</sub> (10%) + 3-4mg drug (Tempol, Chlorambucil-Tempol) in 1ml D<sub>2</sub>O. The hydrogel was prepared by sonicating the mixture using a probe sonicator.
4. NMR relaxation times were recorded on a 400MHz Bruker Avance system with a 5mm QNP probe. <sup>1</sup>H and <sup>19</sup>F T1 relaxation times were measured for the two hydrogel samples
5. ESR spectra for both the hydrogel samples were recorded on a Bruker-EMX EPR system at a field strength of 3490 G.

## RESULTS

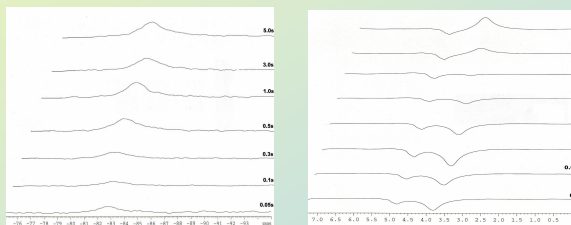


Fig.3 NMR T1 relaxation time spectra using inversion recovery for (a) <sup>19</sup>F terminal groups and (b) <sup>1</sup>H backbone groups spin labeled chlorambucil loaded 10KC8 hydrogel.

Table 1. Comparison of T1 relaxation times for Tempol loaded 10KC8 hydrogel and Spin labeled chlorambucil loaded 10KC8 hydrogel.

Sample	<sup>1</sup> H T1 relaxation times in sec	<sup>19</sup> F T1 relaxation times in sec
10KC8	0.68	0.58
Tempol loaded hydrogel	0.23	0.23
Spin labeled chlorambucil loaded hydrogel	0.70	0.28

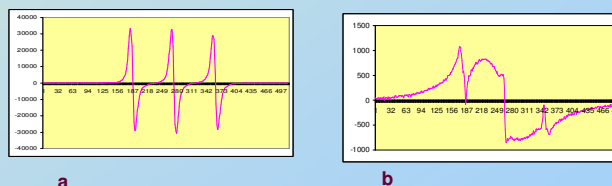


Fig.4 ESR spectra of (a) Tempol loaded 10KC8 hydrogel and (b) spin labeled chlorambucil loaded 10KC8 hydrogel

## DISCUSSION

**Tempol loaded Hydrogel:** The NMR (Fig.3, Table 1) and ESR (Fig4) data reveal that as Tempol has the free hydroxyl group it interacts with the hydrophilic as well as the hydrophobic part of the R<sub>f</sub>-PEG. This leads to spin relaxation of the backbone PEG 1H protons and the terminal <sup>19</sup>F groups. **Chlorambucil-Tempol Hydrogel:** In this case, the drug being hydrophobic it is attracted by the terminal fluorine groups and thus only affects the <sup>19</sup>F relaxation times and thus the backbone 1H relaxation times remain unchanged. This is also proven by the distorted triplet in the ESR spectrum which is partly due to precipitation (broadening) but also reveals interaction with the hydrogel as seen by the sharp peaks.

## CONCLUSION

Evidence that the 2 phase hydrogel systems are good carriers of hydrophobic drugs is seen by this NMR and ESR study.

## FUTURE FOCUS

Continuing studies on the interaction of Tempol labeled drugs in Rf-PEG hydrogels with emphasis on micellar size and number of interacting groups using both NMR and ESR will be dealt with.

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