Polymeric Drugs & Drug Delivery Systems

EDITED BY

Raphael M. Ottenbrite, Ph.D.

Department of Chemistry
Virginia Commonwealth University

Sung Wan Kim, Ph.D.

Department of Pharmaceutics and Pharmaceutical Chemistry
University of Utah

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CHAPTER 20

Examination of Fluorescent Molecules as in situ Probes of Polymerization Reactions

FRANCIS W. WANG[1]
DEBORAH G. SAUDER[2]

INTRODUCTION

PRESENTED in this chapter are the preliminary results of a study designed to examine the feasibility of using a fluorescent dye as an in situ indicator of the physical condition of a bone cement sample. The fluorescence behavior of dyes is often affected by solvent–solute interactions. Variations in solvent dielectric constant, solvent polarity, pH, viscosity, or the presence of hydrogen bonding or other strong intermolecular interactions can all produce substantial changes in fluorescence behavior. Previous studies have illustrated the use of both exciplex [1] and charge transfer, (CT) [2,3,4], probes in monitoring the degree of polymerization in a variety of systems. In the experiments described here we have examined the fluorescence behavior of anthracene and $Re(CO)_3ClL$, (where L = 4,7-diphenyl-1,10-phenanthroline) in commercial bone cements under different concentration and laboratory temperature conditions in which the degree of polymerization is known as a function of time from previous work [5].

The ultimate success of methyl methacrylate bone cements in surgical arenas depends on its application at an appropriate viscosity. Recent studies have raised concerns that the long-term stability of bone cements may be compromised by the empirical way in which the setting of samples is determined [6]. The literature from one manufacturer states that, in addition to the concentration effects one would expect in a biphasic free-radical

[1]Dental and Medical Materials Group, Polymers Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA.
[2]Department of Chemistry and Physics, Hood College, Frederick, MD 21701, USA.

system, ambient temperature and humidity can substantially affect the setting time of a sample. It suggests that ". . . the working time . . . is best determined by the experience of the surgeon . . ."[7]. Farrar and Rose [8] have shown the substantial effects that small ambient temperature variations can have on the dynamic viscosity of a sample of bone cement over time. This study represents an initial effort to understand the behavior of fluorescent probes in methacrylate cements. Eventually, fluorescence may prove useful in providing an in situ quantitative measure of the extent of polymerization of a cement sample by providing a measure of its viscosity.

The bone cement used in these studies was a two-component system. The liquid component [9.75 mL methyl methacrylate (MMA); 0.25 mL *N,N*-dimethyl-*p*-toluidine (DMPT); 75 mg/kg hydroquinone] was mixed with a solid component [3.0 g poly(methyl methacrylate) (PMMA); 15.0 g MMA-styrene copolymer; benzoyl peroxide, mass fraction 2%; 2.0 g $BaSO_4$] to form the cement. Dissolution of the solid component proceeded simultaneously with polymerization once the cement was mixed.

The samples used in this study polymerized via a free-radical, addition mechanism. Although most free radical polymerizations require initiation by the addition of certain labile compounds and/or exposure to heat or light, methyl methacrylate will spontaneously polymerize at room temperature. Hydroquinone is therefore added to the liquid component of the cement to act as an inhibitor — it scavenges the radicals that spontaneously form in the system, limiting polymerization processes during storage. The benzoyl peroxide in the solid component of the cement is present at a sufficiently high concentration that it overwhelms the trace amount of hydroquinone present and acts as a free radical initiator, which is accelerated by DMPT once the solid and liquid cement components are mixed. The $BaSO_4$ serves to make the cement visible via X-ray examination once it is set.

EXPERIMENTAL

The fluorescent probes used in this study were dissolved in the liquid component of the cement before mixing. The probe concentrations were adjusted until a maximum fluorescence emission from the probe dissolved in the MMA liquid component of the cement at room temperature was observed. Anthracene was recrystallized from alcohol before use. $Re(CO)_3ClL$ was synthesized in the manner described by Salman and Drickmar [9]. In this study, samples were prepared for fluorescence measurement by placing 1 to 3 g of the powder component directly in a 1-cm glass fluorescence cell. A known mass of the liquid component (plus probe) was injected into the cell using a syringe and mixed thoroughly with the powder component.

The dissolution of the PMMA polymer and MMA-styrene copolymer in the MMA liquid component occurred simultaneously with polymerization of the cement after mixing. Observations were made on samples maintained at ambient conditions. The presence of $BaSO_4$ in the solid phase rendered the samples opaque, so fluorescence measurements were made over time in front-face reflectance mode. Steady-state fluorescence spectra were obtained using a commercially available spectrophotometer. The bandwidth of the spectrophotometer under experimental conditions was 10 nm. All spectra were taken in ratio mode, so that fluctuations in the incident intensity at the excitation wavelength did not affect the results. No attempts were made to monitor or control the temperature of the system. However, because the volume of sample observed was small, the polymerization was relatively slow, and the sample was in direct contact with the room temperature cell, we did not anticipate that the exothermicity of the polymerization would cause temperature fluctuations large enough to affect the measurements. All intensity values used in the analysis of these results are taken from single-scan, uncorrected fluorescence data. Based on previous experience, we estimate a relative standard uncertainty of ±5% in the peak intensities from single-scan, uncorrected spectra.

RESULTS

As in previous studies [1–4], both substantial spectral shifts and enhancements in fluorescence intensity were observed from the anthracene and Re compound probe molecules as nonradiative energy disposal paths were restricted by the increasing local viscosity accompanying the polymerization processes. New in these studies was the observation that intermolecular quenching, impeded by increasing polymer concentration, allowed the recovery of normal fluorescence to be correlated with a sample's increasing viscosity. The data collected at short times (<5 min) following mixing show no evidence of a red shift, which would be expected if the system dynamics were dominated by local temperature increases due to the exothermicity of the polymerization process.

ANTHRACENE

The intensity of anthracene fluorescence from liquid methyl methacrylate was examined over a wide range of concentrations. The fluorescence intensity measured following excitation at 350 nm was observed to be linear with anthracene concentration up to a mass fraction of 3×10^{-5}% anthracene in MMA. Experimental measurements were made with this concentration of anthracene in the liquid component of the cement. Fluorescence spectra taken as single scans at three different times after mixing a 2:1 powder:liquid (mass ratio) sample are shown in Figure 1. These uncorrected fluorescence spectra have a ±5% relative standard uncertainty in the maximum intensity of the individual fluorescence features. The excitation wavelength was 350 nm. In addition to the sharp features between 370 and 470 nm, which were attributed to fluorescence from isolated anthracene molecules in a polar solvent, there was a broad band centered at ≈540 nm that shifted to the blue as the cure proceeded. In considering previous studies [10] we attributed this band to an exciplex interaction between anthracene probe molecules and the dimethyltoluidine initiator in the cement. In the dilution studies of fluorescence intensity from the liquid component of the cement, the excimer intensity showed a linear dependence on anthracene concentration over the range where the intensity of anthracene monomer feature depended linearly on anthracene concentration.

As expected [2–4], when the solid and liquid cement components were mixed, the anthracene–toluidine complex fluorescence increased in intensity over time as the cure proceeded and nonfluorescence pathways for energy disposal were blocked. Although the change in peak shape made it difficult to comment on the relative fluorescence intensity from the exciplex compared to that from independent molecules, it was clear that the exciplex

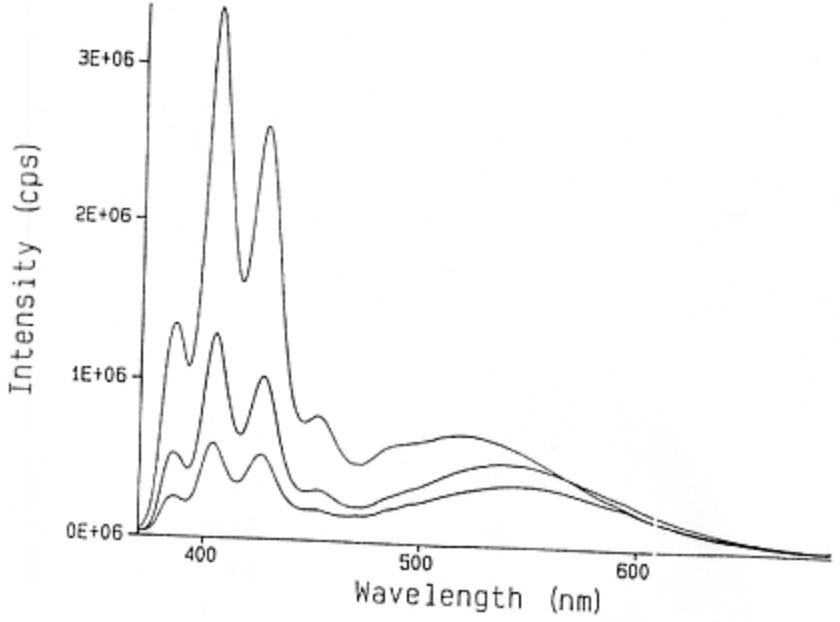

Figure 1 Fluorescence spectra taken as single scans at 3 min (lower curve), 24 min (middle curve), and 121 min (upper curve) after mixing a 2:1 (by mass) powder:liquid sample.

intensity did not increase as substantially with the dissolution/polymerization process as the isolated anthracene fluorescence signal. These results are consistent with a picture that the diffusion of the probe and amine molecules were restricted fairly early in the cure process, thereby limiting the extent of anthracene–toluidine exciplex interaction. In contrast, the nonradiative pathways for exciplex fluorescence continued to decrease as long as the microviscosity continued to increase.

Previous studies [2] emphasized the difficulties in using absolute fluorescence intensity to determine the degree of polymerization. We avoided this problem by using the ratio of intensities of different features that were determined over time. The results from a single sample, followed over time after mixing, are shown in Figure 2. The ratio of fluorescence intensity between two monomer features at 405 and 427 nm remained almost constant over the 2 h time period during which the fluorescence was monitored. The slight decrease in the I(427)/I(405) ratio over the first 20 min of the polymerization was probably due to the wavelength and polarization-dependent transmission efficiency of the emission monochromator because the fluorescence polarization of the sample changed with the increase in viscosity accompanying the polymerization [11].

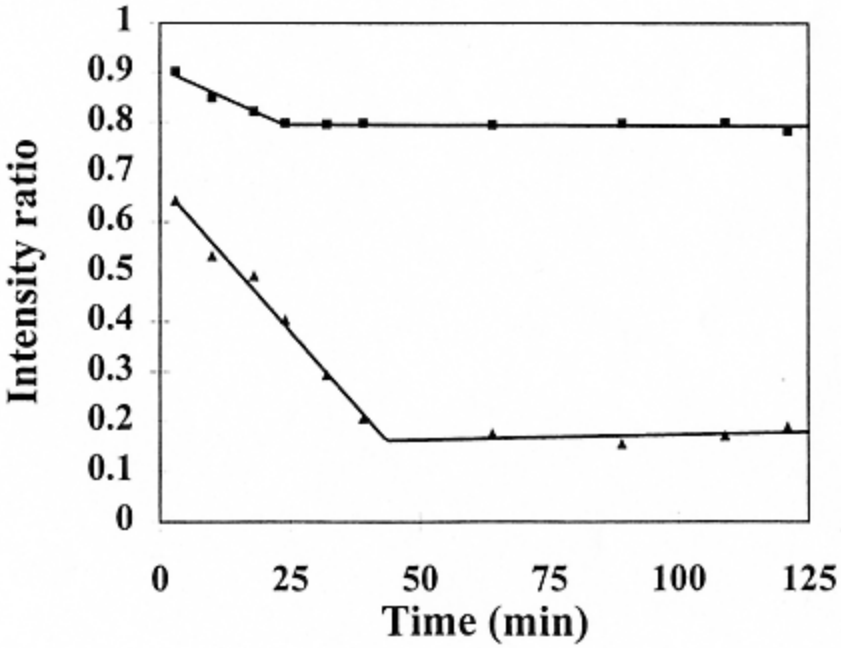

Figure 2 Anthracene monomer, ■ I(427)/I(405) and anthracene–toluidine exciplex to monomer, ▲ I(540)/I(405) peak height ratios from a single sample, scanned repeatedly over the first 90 min of cure.

The exciplex intensity showed quite different behavior as the setting proceeded. A comparison of the (monomer peak/monomer peak) ratio to the (exciplex peak/monomer peak) ratio was quite illuminating. We considered the initial maximum wavelength of the exciplex emission at 540 nm, and compared its intensity to the monomer intensity at 405 nm as the dissolution/polymerization proceeded. A substantial decrease in exciplex intensity, compared to monomer intensity, was observed over the first 40 min of the cure. The ratio then leveled off, indicating that the local viscosity had reached a maximum after 40 min and that the dissolution/polymerization was considered to have reached completion at the ambient temperature of the laboratory. Since the working time for the cement was considerably less than the 40-min time period over which the exciplex/monomer intensity ratio was steadily decreasing, the intensity ratios served *as in situ* monitors of the cure.

RE-COMPLEX

Compounds with the general formula $Re(CO)_3ClL$ are unusual among organometallic compounds in that they photoemit faster than they dissociate under illumination in most solvents over a broad range of temperatures [1]. These compounds also demonstrate a substantial shift in both the wavelength and the intensity of spectral emission in response to microviscosity changes in the solvent [1]. These characteristics made $Re(CO)_3ClL$ complexes particularly attractive as probes to monitor the microviscosity changes that accompany cement cure.

In this study, the complex where L = 4,7-diphenyl-1,10-phenanthroline was used. A solution in which the mass fraction Re-complex in methyl methacrylate was 0.12 % gave two absorption bands at 350 and 475 nm. Both absorption bands produced an emission feature at 612 nm. No other emission features were observed in the visible part of the spectrum.

According to Wrighton and Morse [12], in CH_2Cl_2 the Re-L π^* charge transfer (CT) band is reported at 26,530 cm^{-1} (377 nm) and the intraligand (IL) band is reported at 34,970 cm^{-1} (286 nm). The free ligand has a maximum absorption at 36,760 cm^{-1} (272 nm). In general, the maximum of the lower energy absorption band in these complexes shifts to the blue in more polar solvents. Wrighton and Morse also observed that the emission maximum in $ReCl(CO)_3L$ complexes shifts to the blue upon cooling a sample in EPA to 77 K. Similar studies by Hanna et al. [13], determined that the emission maxima of $ReCl(CO)_3$-2,2′-bipyridine shifted to the blue in both MMA and PMMA solvents when the temperature was reduced from 298 to 20 K.

Our study included only observations of the behavior of the CT band and did not consider the IL band, which should appear farther to the UV than the wavelength range in which these experiments were conducted. In our fluid MMA samples, the CT fluorescence was efficiently quenched,

most likely by the amine accelerator used in the bone cement. However, as dissolution and polymerization proceeded following mixing of the cement components, the viscosity increased, and the Re-complex fluorescence reappeared, shifted to the blue, and increased in intensity. Approximately 15 min after mixing, the Re-complex CT band intensity reached a maximum. Measurements up to 2 weeks after the mixing of the cement showed a constant fluorescence intensity when the cured samples were stored in the dark under ambient laboratory temperature and humidity conditions.

To verify the quenching interaction between the Re-complex and the dimethyl-*p*-toluidine, a Stern-Volmer plot of the results of a concentration dependent study of Re-complex fluorescence intensity as a function of amine concentration in fluid MMA was prepared (Figure 3). The samples contained 1.6×10^{-7} mol Re-complex, and up to a maximum of 2.6×10^{-5} mol of amine, in ≈2.5 g of MMA. Re-complex CT band peak heights at 612 nm were measured from uncorrected fluorescence spectra taken in single scans following excitation at 350 nm. The Stern-Volmer plot is linear over the range of amine concentrations studied. A linear Stern-Volmer plot,

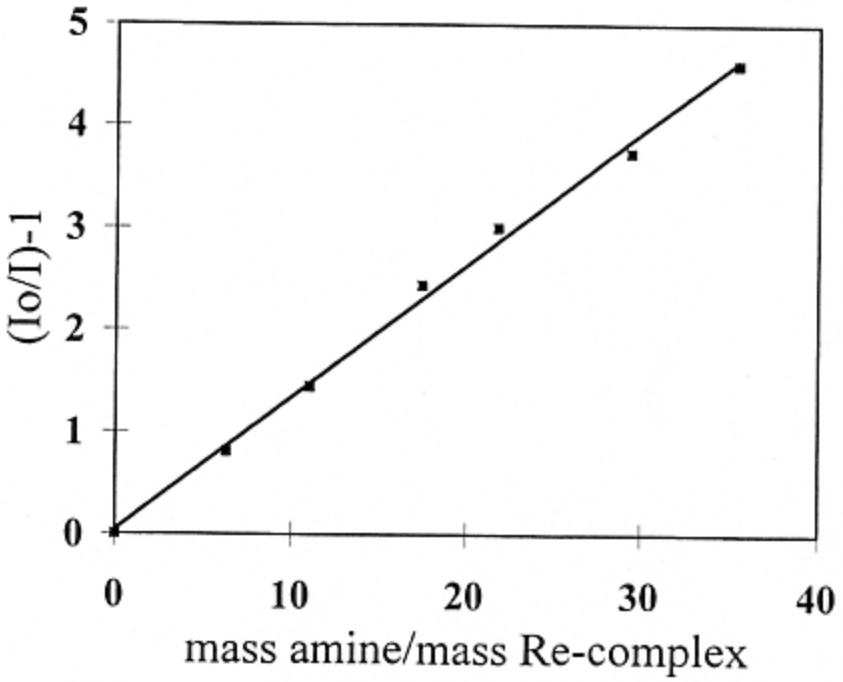

Figure 3 Stern-Volmer plot of the results of a concentration dependent study of Re-complex fluorescence intensity as a function of amine concentration in fluid MMA.

together with the viscosity dependence of fluorescence shown below, indicates that a bimolecular quenching process is occurring. It also supports our assumption that the Re-complex probe is not being degraded by the DMPT on the time scale of interest in these experiments.

In the PMMA environment, as has been seen in previous studies [11], the Re-complex emission was considerably blue shifted. For all the cement samples, the complex was excited at 350 nm, and fluorescence intensity was monitored near 566 nm as a function of time.

The Re-complex fluorescence measured over time from two separate polymerizing samples were analyzed as I(*t*)/I(initial) at 566 nm. I(*t*) is the intensity at 566 nm at some time *t* after mixing of the solid and liquid components of the cement. I(initial) is the CT peak intensity at 566 nm, measured 2 or 3 min after mixing the components, depending on the run. Because the Re-complex intensity did not change in the first 5 min following mixing, the time of the initial reading was not closely controlled. The results from two separate runs showing the evolution of different samples over time are graphed in Figure 4. The fluorescence intensity at 566 nm showed a substantial increase over the time interval from 5 min to 11 min following mixing of the two cement components. A rapid increase in fluorescence intensity occurred during

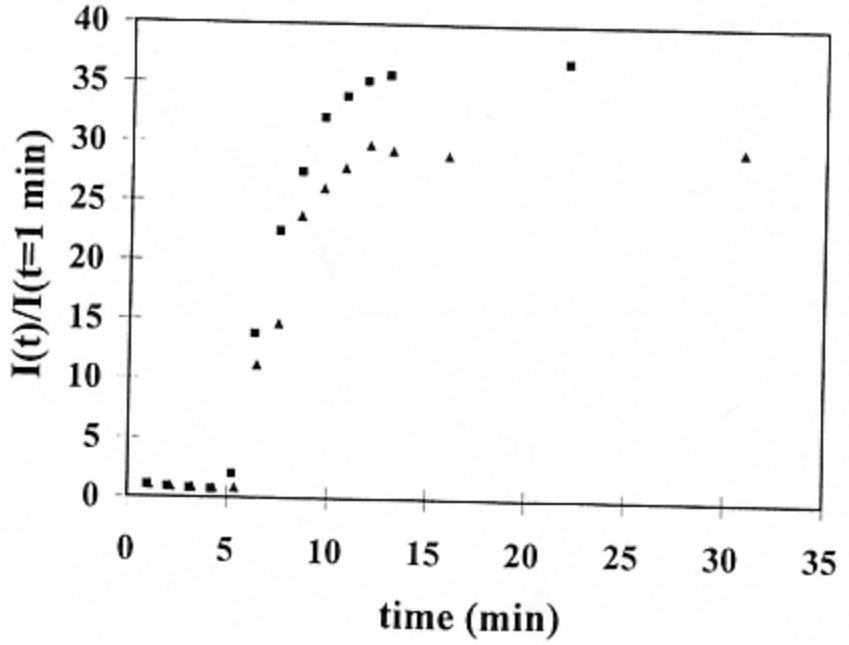

Figure 4 I(*t*)/I(initial) at 566 nm for Re-complex fluorescence measured over time from two separate polymerizing samples.

the time interval in which the cement was expected to reach its working stage as a consequence of the impedance of fluorescence quenching due to the increasing local viscosity. This phenomenon should, therefore, provide a suitable method for in situ monitoring of the viscosity changes that occur during the dissolution/polymerization of PMMA-based cements.

CONCLUSIONS

The results of this preliminary study have shown that anthracene and $Re(CO)_3ClL$ (L = 4,7-diphenyl-1,10-phenanthroline) could be used as in situ monitors of the microviscosity changes that occur as a bone cement sample cured. In addition, the results identified a novel technique—the impedance of quenching—for monitoring local viscosity.

DISCLAIMER

Certain commercial materials and equipment are identified in this work for adequate definition of experimental procedures. In no instance does such identification imply recommendation or endorsement by the National Institute of Standards and Technology or that the material and the equipment identified is necessarily the best available for the purpose.

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