

THE DETERMINATION OF POLYETHYLENE GLYCOL AND WATER IN ARCHAEOLOGICAL WOOD USING INFRARED SPECTROSCOPY AND STEPWISE MULTIPLE LINEAR REGRESSION

Rohan PATEL¹, Jessica BINGHAM¹, Shanna DANIEL²,
Sarah WATKINS-KENNEY², Anthony KENNEDY^{1*}

¹) Department of Chemistry, East Carolina University, East 10th Street, Greenville, NC 27858, USA.

²) North Carolina Department of Cultural Resources, Underwater Archaeology Branch, Queen Anne's Revenge Conservation Laboratory, East Carolina University, West Research Campus, 1157 VOA Site C Road, Greenville, NC 27834, USA.

Abstract

Polyethylene glycol (PEG) is the most common preservative in use for bulking and maintaining structural integrity in waterlogged wood. Conservators therefore have a need to be able to determine PEG concentrations in wood in a non-destructive manner. We present a study highlighting the application of infrared spectroscopy coupled with multivariate analysis techniques to predict the concentration of polyethylene glycol 400 (PEG-400) and water simultaneously. This technique uses attenuated total reflectance (ATR) spectroscopy and unconstrained stepwise multiple linear regression (SMLR) analysis for prediction of multiple components in archaeological wood. Using this model we have calculated the concentration of PEG-400 and water in treated archaeological waterlogged wood samples.

Keywords: *stepwise multiple linear regression; attenuated total reflectance infrared spectroscopy; waterlogged archaeological wood; polyethylene glycol.*

Introduction

Despite recent concerns polyethylene glycol (PEG) is still the most common bulking agent and preservative for treatment of waterlogged archaeological wood. Polyethylene glycol has been commonly employed since it was first recognized as a suitable treatment for waterlogged wood conservation in the late 1950s and early 1960s [1]. Polyethylene glycol, however did not gain popularity among conservators until it was used on a major project to help preserve the wood of the *Vasa*, a 17th century Swedish warship [2]. Polyethylene glycol has also been used and continues to be used for the treatment of other waterlogged 'organic' materials such as leather and textiles [3]. The specific molecular weight polyethylene glycol chosen for a project varies and is dependent on the size and condition of the artifact [4], although one of the most commonly chosen for degraded wood is PEG-400.

Recent problems however with the use of PEG as a treatment include possible degradation of the polymer [5, 6], absorption of water into the artifact and seepage of the

* Corresponding author: kennedyan@ecu.edu, Phone +1-252-328-9816, Fax +1-252-328-6210

polymer from the wood and even more complex reactions if metals are present in the wood [7]. There is clearly a need therefore to be able to determine the relative concentration of polyethylene glycol in wood. One suitable technique involves extraction of the polymer with chloroform [8] and although this method is reliable and performs well it requires precisely controlled conditions and can be time consuming if several samples are to be analyzed. Several instrumental techniques, including X-ray analysis [9] and mass spectrometry [10], have previously been used in the examination of waterlogged wood but again these usually require complex sample preparation procedures or are time and/or sample intensive. A more convenient rapid technique would therefore be highly advantageous.

Complex interactions between PEG-400 and water [11] ensure that spectroscopically the measured absorbance is not linearly related to concentration so a more complex regression technique is required. Multivariate analysis or chemometric techniques have been widely employed to determine the composition of complex samples that are spectroscopically very similar. These methods allow one to analyse several components at once and even to predict physical properties. ATR has already been used previously as a quick method for an estimate of PEG content in treated archaeological wood [11] as well as determining the degree of degradation in the wood [12]. ATR coupled with partial least squares regression analysis has also shown promise for the determination of glucose and lignin in wood [13]. Our study focused on developing a method for the rapid determination of polyethylene glycol and water content in treated archaeological wood. These samples by their very nature are a complex matrix and typically difficult to analyse. Our results however suggest that the model developed is promising and our results are in very good agreement with those obtained using the soxhlet extraction technique. We decided to use the ATR technique for spectral acquisition as it is robust and no sample preparation is necessary reducing complications.

Materials and Methods

Wood Samples

A core wood sample (Oak) was obtained from the hull timbers of the Beaufort Inlet shipwreck (31CR314), Blackbeard's flagship, *Queen Anne's Revenge*. This hull timber had been soaked in tap water for 4 years and then in reverse osmosis water for 1 year 5 months to remove chlorides from the wood. The timber was then placed into a solution of 5% PEG-400 with a polydispersity index of 1.26 (FCC Grade, Dow Chemical Company) in reverse osmosis purified water. After 5 months of soaking the PEG concentration was increased to 20% and the timber was allowed to soak for a further 2 years. The core sample removed for this study was approximately 10cm in length and represented the entire width of the timber. The sample was divided into ten roughly equal segments of approximately 1cm each. The segments were numbered 1 through 10 with segments 1 and 10 containing the wood from either surface of the timber directly in contact with the PEG-400 solution. Segments 6 through 10 were not used in this study. The remaining segments (1-5) were each split into two, one portion being analyzed by gravimetric analysis while the other was used for spectroscopic studies.

Gravimetric Analysis

Samples of the treated wood were slowly dried at 50°C to a constant weight in order to remove water non-aggressively. The PEG-400 was then removed by soxhlet extraction using the method outlined previously [8] until a constant dry weight was obtained. The weight of PEG-400 and water could then be determined. For direct comparison with infrared studies the weights were converted to volumes using the densities of PEG-400 (1.128 g/cm³) and water (0.998 g/cm³) at 20°C.

Infrared Spectroscopy

A series of standards containing PEG-400 and water were prepared. These standards spanned the entire concentration range for each component in 2% increments. Each sample was vortexed to ensure thorough mixing and a homogeneous solution. Spectra of these samples were acquired by obtaining 128 scans at a resolution of 4cm^{-1} in the region $\sim 3700\text{cm}^{-1}$ to 1200cm^{-1} on a Thermo Fisher (Madison WI) 6700 infrared spectrometer using a DTGS detector and a single bounce diamond ATR ($\sim 3\text{mm}^2$). Spectra were then automatically baseline corrected and normalized to set the maximum absorbance to 1. Wood samples were analyzed without any pretreatment. Spectra of the wood samples were obtained and processed in the same manner used for the standards.

Data Analysis

TQ Analyst 8.0 (Thermo Fisher) was used to perform multivariate analysis and build the multiple linear regression models. The previously processed spectra of all the standard solutions were imported directly into the TQ analyst software. Stepwise Multiple Linear Regression (SMLR) was used to build the calibration model and the software was allowed to choose spectral regions to describe both components and also choose suitable validation samples. Two outliers corresponding to 34% and 38% (v/v) PEG-400 were removed and the data was recalibrated. Cross validation was performed by using the leave-one-out method to ensure a suitable model was developed. The model was then used to predict the relative concentration of PEG-400 to water in the wood segments described above. All graphs were prepared using GraphPad Prism 5.

Results

The spectra of the polyethylene glycol and water standards showed several bands that displayed large variations depending on the relative concentration of each component. All spectra were normalized and baseline corrected to simplify the analysis. The water band at $\sim 3500\text{cm}^{-1}$, the C-H stretching vibrations at $\sim 2800\text{-}2900\text{cm}^{-1}$ and the CH_2 wagging band at $\sim 1350\text{cm}^{-1}$ all changed drastically as one would expect (Fig. 1).

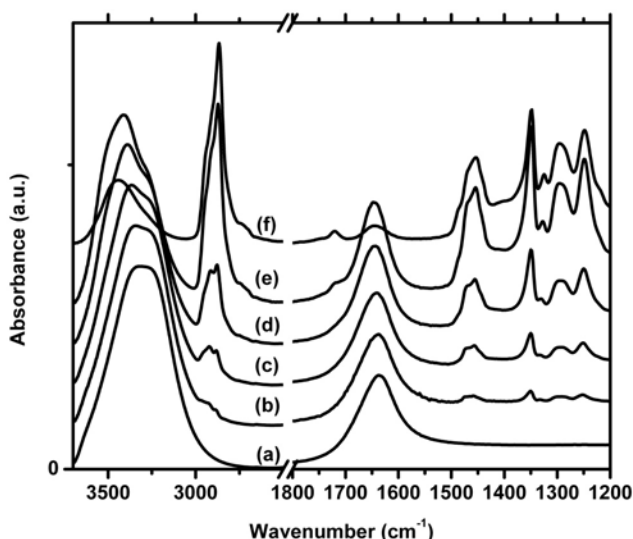


Fig. 1. Representative infrared spectra showing PEG-400 and water standards: (a) 0% PEG-400, (b) 20% PEG-400, (c) 40% PEG-400, (d) 60% PEG-400, (e) 80% PEG-400, (f) 100% PEG-400. Spectra are offset on absorbance axis with the region $2500\text{-}1800\text{cm}^{-1}$ not shown for clarity.

None of these bands however responds linearly to concentration over the entire concentration range for the two component mixture. This is as one would expect as complex interactions introduce large deviations in both frequency and absorbance. The spectra were imported into TQ analyst for modeling. The stepwise multiple linear regression models built from the standard solutions excluding outliers have a correlation coefficient (R^2) of 0.9937 and a root mean squared error of calculation (RMSEC) of 3.37% (Figs 2 and 3).

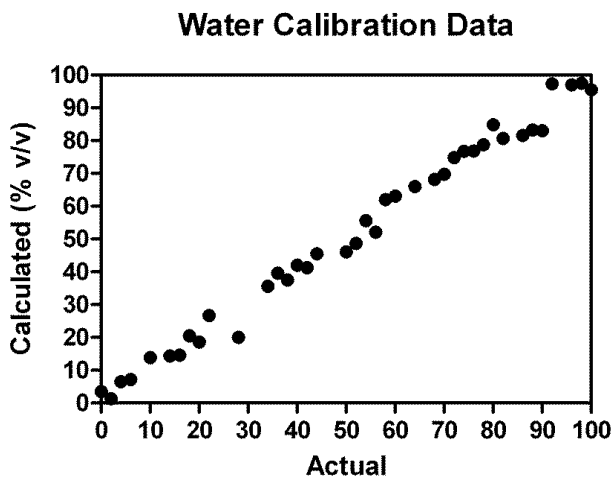


Fig. 2. SMLR model calibration data for water. Correlation coefficient (R^2) 0.9937. Root mean square error of calculation (RMSEC) 3.37%.

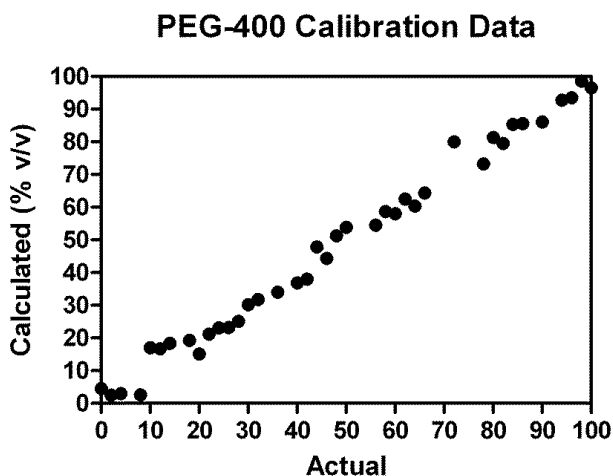


Fig. 3. SMLR model calibration data for PEG-400. Correlation coefficient (R^2) 0.9937. Root mean square error of calculation (RMSEC) 3.37%.

Cross validation gave a correlation coefficient (R^2) of 0.9925 and an RMSECV of 3.67%. The model performed best, based on R^2 and the amount of spectral variation represented, when a sensitive fit value algorithm and mean centering were used. The sensitive fit algorithm measures each validation spectrum relative to the mean spectrum for all calibration standards and determines how well the model performs on validation data. The mean centering technique scales all data prior to calibration by subtracting the average spectrum from each calibration spectrum. Modeling was unconstrained and the program was allowed to choose the

best regions for calibration. The resultant models were used for the calculation of PEG-400 and water concentrations in the wood segments. The data obtained for the archaeological wood samples is shown in (Fig. 4).

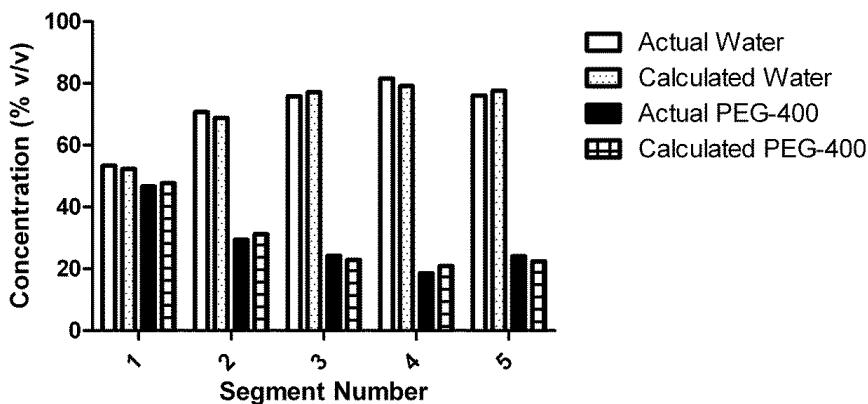


Fig. 4. Actual and calculated PEG-400 and water concentrations (% v/v) for segments 1-5. Actual concentrations determined by gravimetric analysis. Calculated concentrations determined by SMLR model.

Slow drying of the segments followed by soxhlet extraction showed that the PEG-400 concentration as a two component mixture with water varied from ~18% to ~47% (v/v). Analysis of the infrared spectra obtained on the wood samples (Fig. 5) using the previously calibrated SMLR model calculated the PEG-400 concentration to be between ~21% and ~48% (v/v) for segments 1 to 5. The largest discrepancy between the actual and calculated PEG-400 concentration is 2.4% (v/v) and this was found for segment 4. Overall the model performed well and the mean error of prediction for the samples was 1.66%.

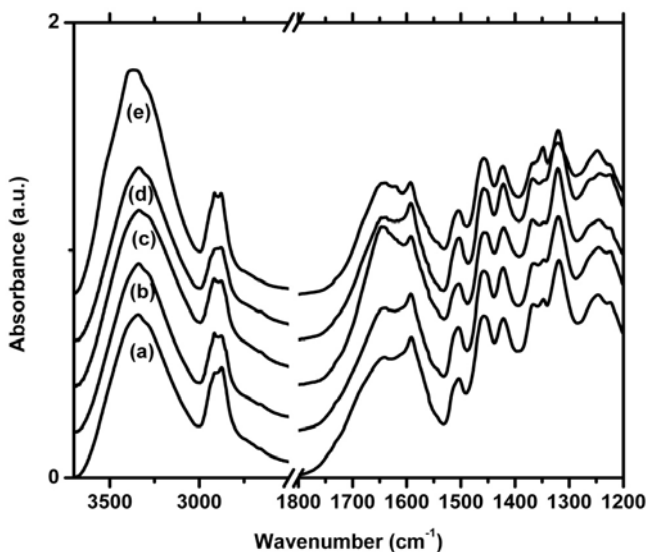


Fig. 5. Infrared spectra of wood segments analyzed: (a) Segment 1, (b) Segment 2, (c) Segment 3, (d) Segment 4, (e) Segment 5. Spectra are offset on absorbance axis with the region 2500-1800 cm^{-1} not shown for clarity.

Discussion

The core wood sample used for this analysis had been soaking in a 20% (v/v) solution of PEG-400 in water. One would naturally expect all of the analyzed samples to contain a final concentration of PEG-400 of approximately 20%. The data in figure 4 clearly indicates large deviations from the expected value although this data is consistent with previous studies on samples obtained from the *Vasa* [6] where PEG concentrations were seen to vary from almost zero to as high as 80%, depending on the degree of degradation in the wood. One can also explain some variation in the ratio of PEG-400 to water due to slow evaporation of water during collection, transport and storage of the samples prior to analysis. In fact this large variation in the PEG-400 concentration may have been beneficial to our study as it allowed us to examine the predictability of the SMLR model over a much wider concentration range than initially planned.

A stepwise multiple regression regression model (SMLR) was chosen as a possible modeling technique as it expresses concentrations based on the absorbance at multiple frequencies and it calibrates each component independently. SMLR may also be used when one can expect to see changes in sample mixture or when the sample matrix contains poorly defined components. This approach seemed particularly suitable to our study as our calibration data would simply be a two component mixture of water and PEG-400 whereas the samples we wished to analyse were far more complex. Wood in itself is a very complex organic matrix that may also include cellulose, hemicelluloses and lignin. With the addition of polyethylene glycol and water the sample matrix gets extremely complex. Variations in the density of the wood would naturally lead to regions or pockets where the local concentrations of polyethylene glycol and water could be very high or low in comparison to the amount of wood. An unconstrained approach was chosen as the regions representing the maximum spectra variation could easily be found in this way. It should be noted however that this approach can be fraught with difficulties [14] especially if the regions are not carefully checked. For this study the regions chosen were clearly inspected before cross validation or prediction to ensure they clearly represented absorption characteristics of the components being investigated and were not subject to large interferences from wood components themselves. Two spectral regions were chosen (2865cm^{-1} and 2808cm^{-1}) by the software to represent and build the calibration curves for both components. The software was also allowed to choose validation samples from within the standard solutions prior to calibration. The correlation coefficients obtained were excellent and we therefore assumed the model had obtained a good fit for the simple two component standards. At this stage we were unsure how well the model would perform on the multi component treated archaeological wood samples but despite these concerns spectra were acquired on wood segments 1 through 5. The samples were simply placed in optical contact with the ATR crystal without any sample preparation or pretreatment and the resulting spectra were baseline corrected and normalized as described previously. We assumed that any liquid in contact with the crystal would be a homogeneous mixture of PEG-400 and water in the wood. The spectra obtained were more complex as we expected due to the inclusion of wood. Despite our concerns the model performed very well and the calculated values of PEG-400 and water were in very close agreement with that obtained from gravimetric analysis and a comparison of the actual and predicted % (v/v) can be seen for both components in Figure 4. Despite the fact that our calibration data covers the entire concentration range we cannot make any conclusions about its ability to predict outside of the range of our samples. Ideally more samples would have to be analyzed for a clearer picture although this is difficult. By their very nature archaeological artifacts are culturally very valuable and although core samples can be replaced, in this instance the gravimetric analysis approach of slow drying and removal of the PEG-400 ensures the wood cannot be placed back into the original timber.

Conclusions

The SMLR model developed was capable of suitably predicting the PEG-400 to water ratio in treated archaeological wood over a wide concentration range. Although the technique is non-destructive it is certainly not non-invasive as a core sample is clearly required at present. However despite this the quantity of sample required for this approach is much smaller than that needed for gravimetric analysis and any core samples could be placed back into the original timber. We expect a similar model would perform equally well with other molecular weight polyethylene glycols. This technique could prove very valuable in determining not only the optimal soaking time for an artifact but also for monitoring PEG degradation and breakdown in treated samples. If an ATR probe were used for spectral acquisition one may be able to conduct studies in a truly non-destructive manner on wood surfaces. We also believe that a similar technique would work for other archaeologically relevant or culturally important samples as long as there is no matrix interference in the specific regions chosen.

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