



Latex quantification in homogenate and purified latex samples from various plant species using near infrared reflectance spectroscopy

Katrina Cornish^{a,*}, Michele D. Myers^b, Stephen S. Kelley^b

^a Western Regional Research Center, USDA-ARS, 800 Buchanan Street, Albany, CA 94710, USA

^b National Renewable Energy Laboratory, 1617 Cole Boulevard, Golden, CA 80401, USA

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Abstract

Parthenium argentatum (guayule) is under commercial development as a source of hypoallergenic latex, which is suitable for the manufacture of latex medical devices safe for use by people suffering from Type I latex protein allergy. Improving agronomic practices, post-harvest shrub handling, and optimizing latex extraction and purification protocols during bioprocessing are hampered by the relatively cumbersome latex quantification methods currently employed. Current methods require several hours before analytical results can be obtained.

The object of our study was to develop and test a near infrared (NIR) spectroscopic method for rapid quantification of latex in both wet and dried *P. argentatum* homogenate and purified latex samples. The procedure was tested on latex samples from *Ficus elastica* (Indian rubber tree), *Helianthus annuus* (sunflower), *Hevea brasiliensis* (Brazilian or para rubber tree) and *Taraxacum kok-saghyz* (Russian dandelion).

P. argentatum latex could be accurately quantified, over a wide concentration range of 0 to 25 mg/ml. The correlations between the measured rubber content and the rubber content predicted by NIR were 0.96 and 0.91 for dry and wet samples, respectively. The presence of homogenate components unrelated to the latex fraction did not perturb the correlations obtained. Similar predictive models could be used to measure the rubber content in *F. elastica*, *H. annuus*, *H. brasiliensis* and *T. kok-saghyz*.

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1. Introduction

All commercial natural rubber comes from a single plant species, the Brazilian or para rubber tree (*Hevea brasiliensis*) and the United States is completely de-

pendent on imports from developing countries (Davis, 1997). Primarily due to its molecular structure and high molecular weight (>1 million Da), natural rubber, cis-1,4-polyisoprene, has high performance properties that cannot be matched by synthetic rubber produced from petroleum. The *H. brasiliensis* crop consists almost entirely of plantation-grown clonal trees, and is arguably one of the world's most genetically-narrow crops. This lack of genetic diversity leaves the

* Corresponding author. Tel.: +1-510-559-5950;

fax: +1-510-559-5663.

E-mail address: kcornish@pw.usda.gov (K. Cornish).

crop susceptible to pathogenic attack and failure, as demonstrated by the devastation caused by leaf blight in South American plantations (Davis, 1997). A dependable rubber supply is endangered by many other factors, including diminishing acreage as growers in developing countries move away from rubber farming toward higher value agriculture, increasing global demand, and changing political positions (Davis, 1997; Reisch, 1995). Also, *H. brasiliensis* climatic requirements limit its cultivation to specific tropical regions. Thus, commercially-viable rubber-producing crops suitable for cultivation in temperate regions are greatly desired.

Although some 2500 plant species produce natural rubber (Bowers, 1990; Ray, 1993) many are tropical and most do not produce the high molecular weight polymers required for high quality commercial products where molecular weight is strongly correlated with rubber quality (Swanson et al., 1979). Of the possible rubber-producing plant species for the United States, *Parthenium argentatum* (guayule), a woody desert shrub (Whitworth and Whitehead, 1991), has received the most sustained research and development effort over the years and is closest to domestication and commercial production. Currently, *P. argentatum* is being introduced as a biennial crop in the southwestern United States to supply high performance hypoallergenic latex to the medical products market (Carey et al., 1995; Siler and Cornish, 1994; Schloman et al., 1996; Cornish, 1996; Siler et al., 1996; Cornish, 1998; Cornish and Lytle, 1999). To expedite the commercialization effort, factors affecting the yield and stability of latex, during cultivation and in harvested shrub prior to processing, must be understood (Cornish et al., 1999, 2000, 2001). These factors can then be used to optimize and establish agronomic management practices and post-harvest storage conditions for the shrub in order to maximize latex yield (Cornish et al., 2000). More rapid latex quantification methods, than those currently in use (Cornish et al., 1999), are greatly needed to help achieve these goals.

Although *P. argentatum* should be able to fully supply the medical products market, it cannot tolerate the extended snowy winters and severe temperatures experienced by much of the United States. The development of alternative annual rubber-producing plants, such as *Helianthus annuus* (sunflower), should allow

rubber-production over a much larger acreage, provide for domestic non-medical rubber production requirements, and lead to rubber exports. Development of *H. annuus*, and other annual crops, is hampered by the relatively cumbersome latex quantification methods currently employed especially at the very low latex concentrations contained within existing sunflower lines (0.1–1%, compared with 10% in *P. argentatum*, on a dry weight basis (Cornish et al., 1999)). Current methods require several hours before a quantification result can be achieved.

Near infrared (NIR) spectroscopy has been used for the characterization of different forms of biomass for more than 15 years (Marten et al., 1985). The NIR portion of the spectrum ranges between 750 and 2500 nm, just above visible red. Absorption in this range provides information on overtones of the bending and stretching vibrations of C–H, O–H, and N–H bonds, making the information in the spectrum useful for analyzing the individual components in biological materials (Marten et al., 1985).

A number of spectroscopic tools, including NIR, have been used to study the composition of synthetic polymers including butadiene and isoprene rubbers (Fraga, 1959; Black et al., 1985; Marinho and Monteiro, 2000; Guilment and Bokobza, 2001). These studies have highlighted the value of using NIR for quantitative measurements of rubber properties including the rubber content in biomass (Kleine and Foster, 1990), and the ratio of *trans* to *cis* in rubber mixtures and copolymers (Marinho and Monteiro, 2000; Guilment and Bokobza, 2001). A recent study by Guilment and Bokobza (2001) compared Raman, mid-range IR, and NIR, which showed the power of using chemometric analysis tools for measuring the *trans*, *cis* and vinyl content of butadiene copolymers. Also, the *trans* to *cis* ratio in mixtures of rubber from *H. brasiliensis* and *Mimusops globosa* or *H. brasiliensis* and gutta-percha could be accurately measured with NIR, although there were significant non-linearities in other mixtures, such as *Ficus elastica* and gutta-percha (Marinho and Monteiro, 2000).

Early work by Black et al. (1985) showed that NIR could be used to measure the rubber and resin concentration in guayule. However, the sample had to be slowly and carefully ground to obtain a very uniform substrate. Non-uniform samples resulted in

poor reproducibility. This work also focused on one or two individual wavelengths rather than using the entire NIR spectrum as is done in this work. In a subsequent study on the use of NIR and chemometric tools, Kleine and Foster (1990) showed that both the rubber and resin content could be measured directly and accurately on dried, finely ground, guayule plants. This technique has been used to assist with screening of different lines of guayule grown under different conditions, or from direct-seeding or transplantation (Foster et al., 1999, 2002). As with the earlier work by Black et al. (1985), analysis required a time-consuming grinding process to achieve the homogenous substrate required for the production of high quality predictive models. A NIR measurement can be complete in less than one minute, which is much faster than a traditional gravimetric method that requires time-consuming extraction and drying.

In this paper, we show that NIR and chemometric data analysis tools can be used to quantify natural rubber content from a range of different sources, in aqueous homogenates, which can be made from plant samples in a few minutes, and in purified lattices.

2. Materials and methods

2.1. Plant material

2.1.1. *Parthenium argentatum*

One liter of clarified *P. argentatum* homogenate was prepared from stem bark of 5-year-old plants of line AZ-R2 as described (Cornish and Brichta, 2002), although these techniques should work equally well for other lines. The 21 × 45 ml samples (in 50 ml Falcon tubes) were centrifuged at 2000 × g in a bucket rotor for up to 10 ×. Different latex concentrations in clarified homogenate samples were prepared as follows. The lower concentrations were prepared by removing different amounts of the latex that floated to the tops of the tubes and then re-mixing each homogenate. Higher concentrations were made by adding latex to tubes of the original homogenate to make higher concentrations than in the starting homogenate. This procedure was repeated until a wide concentration range was obtained, including a sample from which all the latex had been removed. Most of the floated latex was removed and pooled for later purification (see next para-

graph). Latex concentrations were determined using established procedures (Cornish et al., 1999) where 3 × 1 ml aliquots were centrifuged, the floated latex was coagulated with glacial acetic acid, the aliquots were re-centrifuged, and the coagula removed, dried and weighed. After quantification, some homogenate samples were re-centrifuged and latex transferred to other tubes to improve the range of latex concentrations among the samples between 0.13 and 36 mg/ml. Final latex concentrations were determined in a similar manner.

P. argentatum latex was purified from the latex harvested, as described previously, and purified five times with 0.05% ammonium alginate creaming agent (0.1% Collatex A/RN alginate by Kelco) in aqueous 0.2% NH₄OH and 0.1% Na₂SO₃ (AAO) as described (Cornish and Brichta, 2002). The latex concentration was determined (Cornish et al., 1999) as 198 mg/ml. Pure latex samples of different concentration were made by dilution with the creaming agent buffer, to generate samples between 0.10 and 40 mg/ml, as well as buffer alone.

2.1.2. *Ficus elastica*

Latex was harvested from greenhouse-grown plants in Albany, CA, and purified in AAO by centrifuging for 10 min at 2850 × g in a bucket rotor. The sedimented rubber particles were suspended and re-centrifuged four times to remove soluble components from the latex. The latex was re-suspended the final time in 0.05% ammonium alginate in AAO.

2.1.3. *Helianthus annuus*

Sunflower plants of line 4049 (Interstate) were grown in a field performance test conducted at the Western Colorado Research Center at Fruita, CO during 2001. Plants were harvested and shipped overnight in coolers with ice to Albany, CA. Leaves were excised from the plants and ground in a 4 l Waring blender on low for 1 min in AAO (1:1, w/v). 1:1 (w/v). The homogenate was filtered through four layers of grade 60 cheese cloth. The filtrate was adjusted to pH 10 with NH₄OH and then to 200 mM EDTA. The homogenate was centrifuged for 25 min at 5850 × g in a bucket rotor. The floated latex layer was scooped off into 5 ml of AAO and purified by repeated creaming in 0.1% ammonium alginate as described for *P. argentatum* latex (Cornish and Brichta,

2002). Creaming was repeated twice beyond when the supernatant solution became colorless.

2.1.4. *Hevea brasiliensis*

H. brasiliensis latex was purified twice using 0.1% ammonium alginate creaming agent and then twice with 0.05% as described for *P. argentatum* latex (Cornish and Brichta, 2002).

2.1.5. *Taraxacum kok-saghyz*

Rubber latex was extracted and purified from the roots of hydroponically-grown *T. kok-saghyz* in a manner similar to that described for *H. annuus*, except that a 1 l Waring blender was used.

2.2. Preparation of latex/paper samples

Because one of the goals of this work was to develop a quick and easy assay for rubber in *P. argentatum* plants, samples were prepared to emulate the plant system. Samples were prepared by pipetting 0.5 ml of clarified homogenate or purified latex onto a thick piece of highly bleached cellulose blotter paper (Rayonier Inc.), to create a rubber/cellulose composite sample about 4 mm in diameter. The properties of the cellulose substrate are not important as long as there is little sheet-to-sheet variation. This rubber/cellulose composite sampling protocol also eliminated the opacity problems associated with using a transmission NIR protocol to collect spectra on a latex suspension (data not shown). This simple rubber/biomass composite sample protocol was seen as a rapid, accurate technique for either laboratory or process control in industrial applications. Two to four separate 4 mm diameter rubber/cellulose composite samples were prepared from each sample. The rubber/cellulose composite samples were allowed to air dry for 30 min and their NIR spectra were collected. This set was designated as the “wet samples”. The rubber/cellulose composite samples were then air dried for 24 h and the NIR spectra collected again. This set of samples was designated as the “dry samples”.

2.3. Near infrared spectroscopy

The NIR measurements were all made with an Analytical Spectral Devices (ASD, Boulder, CO) Field Spec Pro at wavelengths between 1000 and 2500 nm.

The visible region was tested but then excluded from the subsequent analyses to eliminate the clear differences in color between the latex and extracted samples. A fiber optic probe oriented at a right angle to the sample surface was used to collect the reflectance spectra from a 3 mm diameter sample area. A piece of commercial microporous Teflon was used as the white reference material. The samples were illuminated with a dc lamp (Lowepro, Santa Rosa, CA) oriented at 30° above the samples. Thirty individual scans were collected, at 1 nm intervals, from each sample and averaged into a single spectrum. The averaged reflectance spectra were transferred from the ASD and converted to absorbance spectra in Unscrambler® (CAMO, Inc., Corvallis, Oregon). The 1 nm intervals were averaged to generate a spectral data set at 10 nm intervals. Such averaging of the spectral data both reduces the size of the spectra matrix and significantly reduces the time required to compute the projection to latent structures (PLS) models without decreasing the quality of the models. This averaging also suggests that spectra gathered with a lower resolution spectrometer would give comparable results. Spectra from each of the two to four replicate “spots” were subjected to a preliminary principal component analysis (PCA) to identify outliers or anomalous samples. No unusual samples were found and all of the replicates then were averaged into a single spectrum for the sample. This single spectrum was then used to predict the rubber concentration of the sample.

2.4. Multivariate analysis (MVA)

Multivariate analysis was performed using The Unscrambler® version 7.6 (CAMO, Corvallis, OR), capable of PCA and PLS analyses. Several different preprocessing techniques, e.g., second derivatives or multiplicative scattering correction, were evaluated for their effects on the quality of the PLS models. Both preprocessing techniques are commonly used to reduce variations due to baseline shifts or curvature (Lavine, 2000; Ozaki et al., 2001).

2.4.1. PLS analysis

For the PLS models, all the NIR spectra were combined into a single data matrix (X-matrix) whereas the rubber content data was combined into a response vector (Y-matrix). Both the X- and Y-matrices were

mean-centered variance normalized prior to performing the PLS analysis. The number of principal components (factors) used for a model were selected by observing response of the residual Y-variance with added factors. Iterations were stopped when additional factors did not substantially decrease the residual Y-variance. All of the PLS models reported here are based on two to four latent variables.

The calibration models (CALB) for the spectra of the “dry” samples were constructed with one-half of the samples (38) using full cross-validation. Cross-validation systematically removes a single sample from the data set, constructs a model with the remaining samples and uses that model to predict the rubber concentration of the removed sample. This process continues until each individual sample has been removed from the data set and a fully cross-validated model was constructed (Martens and Naes, 1991). This fully cross-validated model was then used to predict the response of a test set (TEST) that contained the other one-half of the samples (37) that were not included in the original model. This conservative approach insures that the predictive capabilities of the model are reliable. Models used to predict the rubber concentration in *F. elastica*, *H. annuus*, and *H. brasiliensis* did not have enough samples to construct a separate TEST set so that the values are based on predictions calculated from the fully cross-validated model alone. The results from these small sample sets should be viewed as an indication of the potential of the analysis.

Analysis of the spectra of the wet samples involved dividing the samples into three groups, each containing at least 15 samples with rubber concentrations between 0 and 10 mg/ml. The first group was used to construct an orthogonal signal correction (OSC) calibration model (Fearn, 2000). This OSC was used to minimize the vibrations due to water. OSC essentially removes the vibrations of water by identifying spectral features that are completely non-correlated with the response of interest, e.g., rubber content, and removing those spectral features. A standard PLS model can then be constructed from the OSC corrected spectra. This OSC calibration model was applied to the two remaining groups. The second group, containing OSC corrected spectra, was then used to construct a fully cross-validated PLS CALB model. Finally the third group was used to test the PLS model.

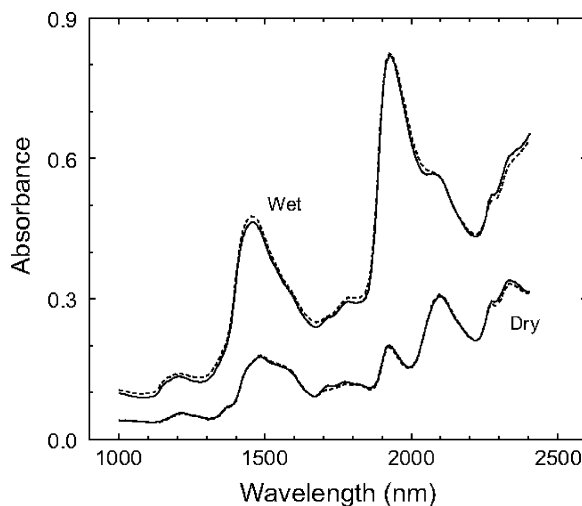


Fig. 1. The NIR spectra of wet and dry samples of cellulose/rubber (*P. argentatum*) composites after multiplicative scattering correction. Two spectra of wet cellulose/rubber composites overlap, as do the two spectra of dry cellulose/rubber composites with one of dry cellulose (all three spectra are highly overlapped indicating the need to detect very subtle differences).

3. Results and discussion

3.1. NIR spectra of rubber/cellulose composites

The NIR spectra of the original dry and wet samples (prepared as described in Section 2.2) are shown in Fig. 1. The lower spectrum of dry cellulose, without any rubber, is dominated by the vibrations associated with cellulose (Ali et al., 2001). These vibrations include major OH overtones and combination vibrations at 1500, 1924, and 2100 nm. There is no discernable difference between the spectra of the dry cellulose and dry rubber/cellulose composites (third and fourth spectra from the top). This is not too surprising because the actual rubber concentration in the area analyzed with NIR is quite low, latex sample L01-D with 18.0 mg of rubber and homogenate sample E02-D with 18.4 mg. The spectra of the wet samples (the upper two spectra) are dominated by water-associated vibrations at 1450 and 1924 nm. These original spectra show little evidence of CH₂ or CH₃ vibrations at 1417, 1720, and 2200–2350 nm that are indicative of natural rubber (Black et al., 1985; Kleine and Foster, 1990; Marinho and Monteiro, 2000; Guilment and Bokobza, 2001). The lack of clear spectral evidence highlights the need

for, and value of, MVA tools such as PCA and PLS to study these rubber/cellulose composites.

3.2. Near infrared analysis of *P. argentatum* homogenate and latex

3.2.1. Principal component analysis of dry *P. argentatum* homogenate and latex

The NIR spectra of the homogenate and purified latex were subjected to PCA in an attempt to differentiate the two sets of samples. The homogenate samples have an opaque greenish or reddish brown color, whereas the latex samples are milky white. Because the colors of these two sample sets are distinctly different, a spectral range of 1000–2400 nm was used to eliminate the impact of color on this analysis. Even so, PCA analysis demonstrates clear differences between the two sample sets (Fig. 2), probably caused by the presence of the soluble plant components, such as tri- and diterpenoid resins and protein, which are known to be present in the homogenate but removed by latex purification. Each principal component (PC) contains different information on the chemical features that differentiate the samples (Fig. 3). PC1 contains 68% of the spectral variation and PC2 contains 15% of the spectral variation. The spectra are essentially a mirror image of one another between 1870 and 2170 nm indicating that the samples are different in

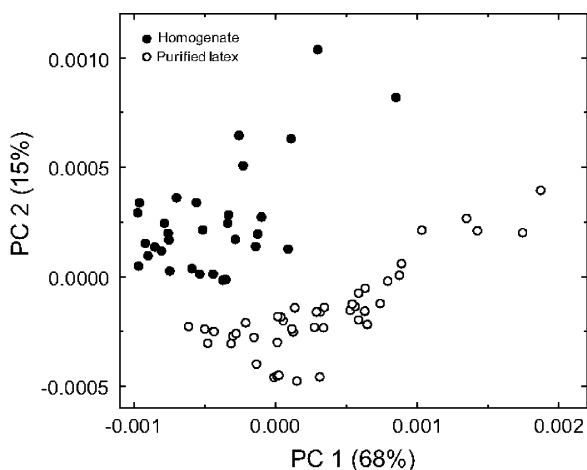


Fig. 2. Results of PCA analysis of homogenate and purified latex samples from *P. argentatum*, from the second derivative of NIR spectra.

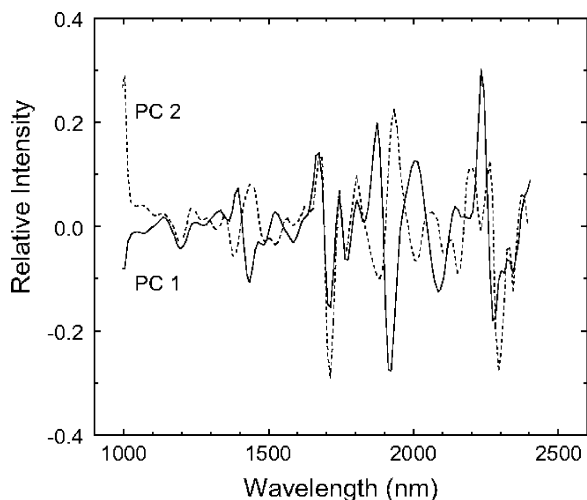


Fig. 3. Loadings for PC1 and PC2 that show the chemical features that differentiate homogenate and purified latex samples.

this range (Fig. 3), and the PCs also differ between 2170 and 2320 nm. However, despite these clear spectral differences, the PCA analysis (Fig. 2) reveals that both PC1 and PC2 contain information related to the concentration of rubber in the sample, with samples in the upper right-hand side having a high concentration of rubber, whereas those in the lower left-hand corner have a low concentration. The strong vibrations at 1710–1720 nm, in both PCs (Fig. 3) are related to the rubber concentration.

3.2.2. Projection to latent structures analysis of dry *Parthenium argentatum* homogenate and latex

The NIR spectra of the dry samples were also subjected to PLS analysis to quantify their relationship to the rubber concentration in the samples (Fig. 4). Both the homogenate and latex samples were included in the PLS model to test the reliability of NIR for measuring rubber concentration. There is a strong correlation between the measured and predicted rubber concentration for both the CALB ($r = 0.95$) and TEST ($r = 0.96$) sets. This suggests that the models are accurate and can effectively predict the rubber concentration of unknown *P. argentatum* samples. The ability to predict the rubber concentration in both the homogenate and latex samples with only one model also shows that this correlation is based on the spectral features of the rubber molecule, and that secondary components

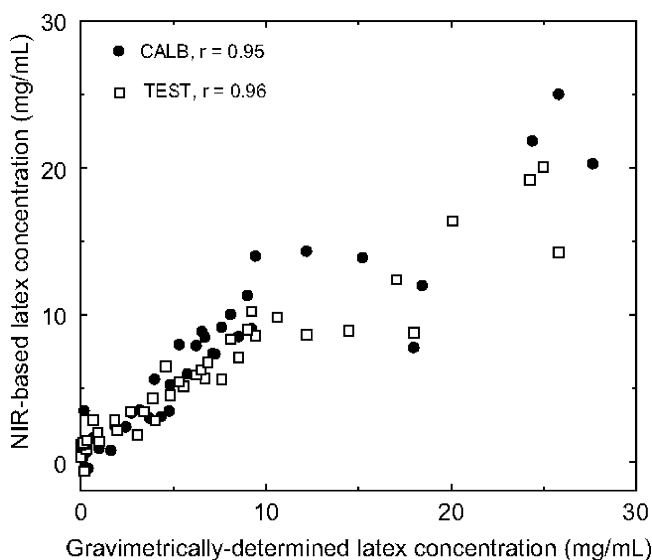


Fig. 4. Measured rubber concentration and rubber concentration predicted from NIR spectra of dry samples: (●) samples in the CALB set and (□) samples in the TEST set.

noted earlier (Fig. 2) do not have a significantly negative impact on the rubber correlation. Construction of PLS models from only latex samples or only homogenates (not shown) did significantly change the correlation between the measured and predicted rubber content (see Table 1). The root mean square error of prediction (RMSEP) for the homogenate samples is slightly better than that of the combined set or the latex only set, but the models are all very similar.

To test the effectiveness of this NIR/MVA technique for measuring rubber content at low concentrations, all samples with less than 10 mg rubber/ml were used to construct two additional PLS models. In this case, the homogenate and latex samples were separated and each set was used to construct a PLS model (Fig. 5). As seen for the combined data of higher rubber

concentrations (Fig. 4), the correlations between predicted and actual rubber concentration are very strong for both the homogenate and latex. The efficacy of the NIR method at these low concentrations would be very useful in bioprocess control applications.

The regression coefficients for the PLS models shown in Figs. 4 and 5 represent the chemical properties responsible for the correlations (Fig. 6a–c). All three sets of regression coefficients are quite similar, indicating that the same chemical (spectral) features are responsible for the correlations. All three have strong absorbance features at 1715 and 2295 nm consistent with chemical features associated with the CH=CH, CH₂ or CH₃ vibrations of natural rubber (Black et al., 1985; Kleine and Foster, 1990; Marinho and Monteiro, 2000; Guilment and Bokobza, 2001).

Table 1
Properties of PLS models constructed for different rubber samples

Rubber source	Correlation coefficient	Root mean square error of prediction	Concentration range (mg/ml)
All <i>P. argentatum</i>	0.96	2.7	0–35
<i>P. argentatum</i> (homogenate)	0.86	1.4	0–10
<i>P. argentatum</i> (latex)	0.96	1.2	0–10
Generalized model	0.82	4.5	0–35

These models were constructed using a separate TEST set to evaluate the original PLS model.

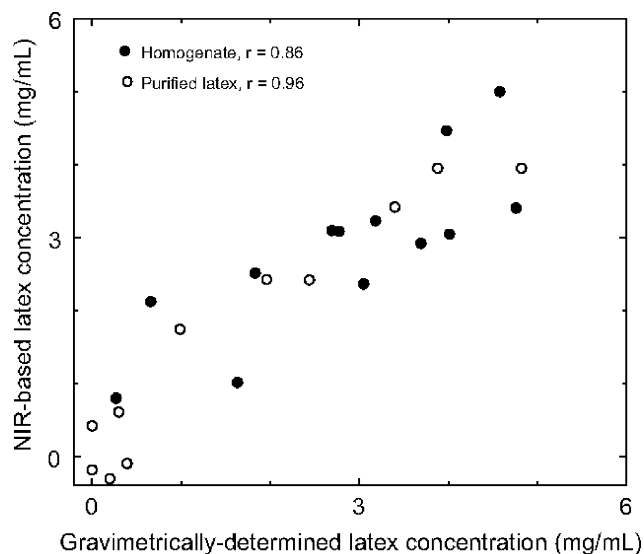


Fig. 5. Measured rubber concentration (<10 mg/ml) and rubber concentration predicted from NIR spectra of dry samples: (○) are purified latex sample and (●) homogenate samples.

Specifically, the major peaks centered around 1720 and 2310 nm can be assigned to different methyl or methylene combinations or overtone vibrations (Kleine and Foster, 1990; Marinho and Monteiro, 2000; Guilment and Bokobza, 2001). Between 1850 and 2180 nm, the regression coefficients for the homogenate are slightly different than those of the combined set or the latex alone.

3.2.3. PLS analysis of wet *P. argentatum* rubber samples

The NIR spectra of wet *P. argentatum* samples are dominated by the vibrations associated with free water (Fig. 7). The two major peaks at 1450 and 1920 nm can be assigned to the first overtone of free water and carbohydrate hydroxyls, and to the interaction of bound water with carbohydrate hydroxyls,

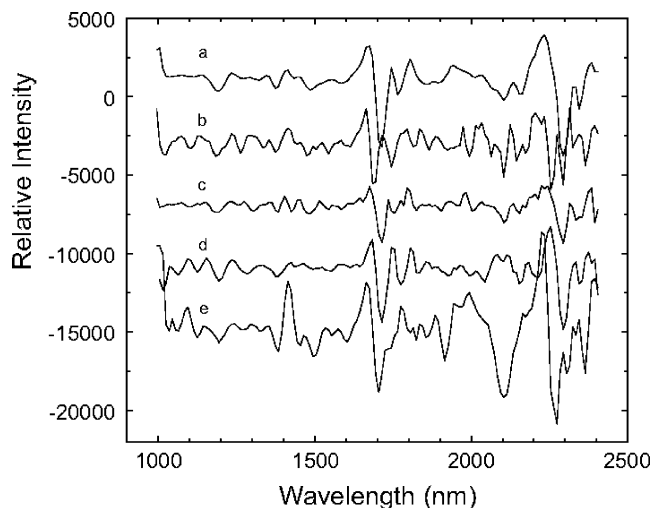


Fig. 6. Regression coefficients for PLS models shown in Figs. 4 and 5: (a) all *P. argentatum* in the 0–35 mg range, (b) *P. argentatum* purified lattices (0–10 mg/ml), (c) *P. argentatum* homogenate (0–10 mg/ml), (d) wet *P. argentatum* samples (0–10 mg/ml), and (e) generalized model.

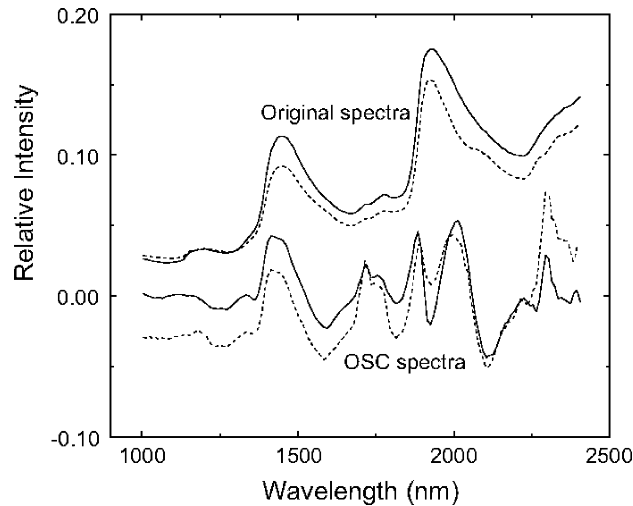


Fig. 7. NIR spectra of the wet cellulose/rubber composites before and after orthogonal signal correction. PCA analysis of wet samples from *P. argentatum*.

respectively. There are no spectral features that can be readily assigned to *P. argentatum* rubber, or even spectral features associated with the cellulose substrate. Therefore, to highlight the spectral features associated with the *P. argentatum* rubber, OSC was applied to the wet *P. argentatum* samples. The OSC essentially “drys” the samples by removing spectral features, such as the water signal, not correlated with the response of interest, i.e. rubber concentration in this case. The two OSC-corrected spectra (Fig. 7) clearly show that the strong vibrations of water at 1450 and 1920 nm have been removed from the spectra, and there are now distinct vibrations at 1720 and 2300 nm associated with the rubber component.

Twenty-six OSC corrected samples then were subjected to PLS analysis, 13 (CALB) being used to construct a PLS model, whereas the remaining 13 (TEST) were used to evaluate the model (Fig. 8). The correlation coefficients for both the CALB ($r = 0.91$) and TEST ($r = 0.90$) models are high. Both the original PLS model and the predictions used four latent variables, which is more than were used with the PLS models for the dry *P. argentatum* spectra. However, the strong correlation of the TEST set indicates that the model is not “overfitting” the data. The need for the additional latent variables is likely due in part to the limitation of the OSC model used to correct for the moisture content of the wet spectra, and relatively low

rubber concentrations in the samples used to develop the model.

The regression coefficients for the PLS model of the wet *P. argentatum* samples (Fig. 6d) are very similar to those produced for the PLS models of the dry samples shown in Figs. 4 and 5 (Figs. 6a–c). Again, the regression coefficients for the PLS model of the wet *P. argentatum* samples have strong spectral features at 1715 and 2295 nm consistent with the CH=CH, CH₂ or CH₃ vibrations of natural rubber (Black et al., 1985; Kleine and Foster, 1990; Marinho and Monteiro, 2000; Guilment and Bokobza, 2001). The similarities between the regression coefficients for the dry and wet PLS models are significant for two reasons. First, they confirm that the same NIR vibrations are dominating the correlations for both dry and wet samples, and these vibrations can be assigned to natural rubber. Second, the similarities confirm that OSC effectively removed the spectral features associated with water, without removing the essential spectral features associated with the natural rubber.

3.3. Analysis of rubber from other plant species

To test the potential for using NIR to measure the characteristics of natural rubber isolated from other plants, a series of latex samples from *F. elastica*, *H. annuus*, *H. brasiliensis* and *T. kok-saghyz* were

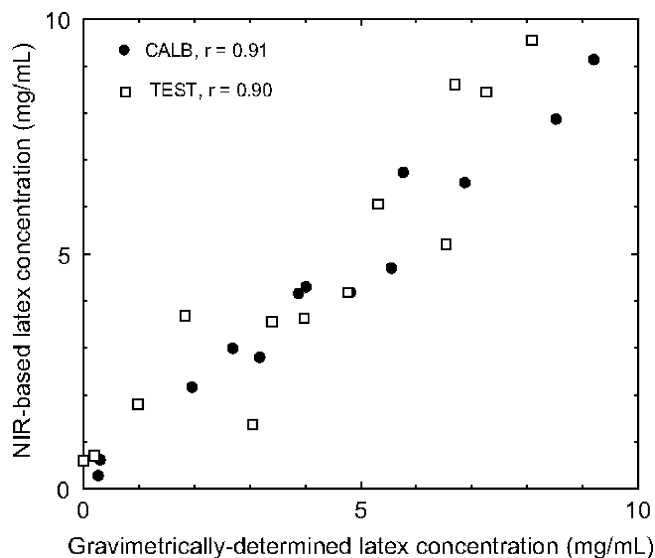


Fig. 8. Measured rubber concentration and rubber concentration predicted from NIR spectra of wet samples: (●) samples in the CALB set and (□) samples in the TEST set.

analyzed. All of these samples were analyzed as purified lattices using the same techniques as for the *P. argentatum* rubber latex. The spectra of all the samples (not shown) were similar, again emphasizing the need for the MVA tools.

3.3.1. Principal component analysis

As seen for the *P. argentatum* samples, the greatest variation in the spectra is due to differences in the rubber concentration of the sample. To minimize these differences, samples with concentrations between 8 and 12 mg/ml were used in the PCA. This selection criterion resulted in only a few rubber samples from each species, except *P. argentatum*.

PC1 was dominated by spectral features that could be assigned to cellulose, and appeared to be due to non-uniform coverage of several rubber samples on the cellulose substrate. However, PCA indicates that the natural rubbers isolated from different sources can be distinguished from one another, and suggest that it may be possible to quantify the rubber content in all these species. For example, the plot of PC3 against PC2 (Fig. 9a) shows that *P. argentatum* and *T. kok-saghyz* lattices are quite similar along PC2 and PC3, whereas *H. brasiliensis* and *H. annuus* lattices are clearly differentiated along PC3. In contrast, when PC3 and

PC4 are compared (Fig. 9b) the *P. argentatum* and *T. kok-saghyz* lattices differentiated, whereas the *H. brasiliensis* and *H. annuus* rubbers are similar.

The chemical loadings, or contributions, for this PCA (Fig. 10), must be interpreted with care because of the small sample numbers used. The loadings for PC2 suggest that one main feature separates *H. brasiliensis* and *F. elastica* that is consistent with protein/water interactions. These samples may have dried more slowly than the others or they may contain more protein. The loadings for PC3 and PC4 have well-defined features at 1720 and 2300 nm that can be assigned to vibrations in natural rubber. PC3 also has a very strong feature at 2340 nm and a shoulder at 1760 nm that could be due to other hydrocarbons, e.g., resins that are present in *P. argentatum* and can be detected by NIR (Black et al., 1985).

3.3.2. PLS analysis

There were insufficient samples to construct PLS models for the wet samples. The wet sample required the use of OSC and about one-third to one-half of the samples are required to construct the OSC model.

However, the concentration of rubber in the dry samples could be estimated with a "generalized" PLS model. To prevent the *P. argentatum* samples from

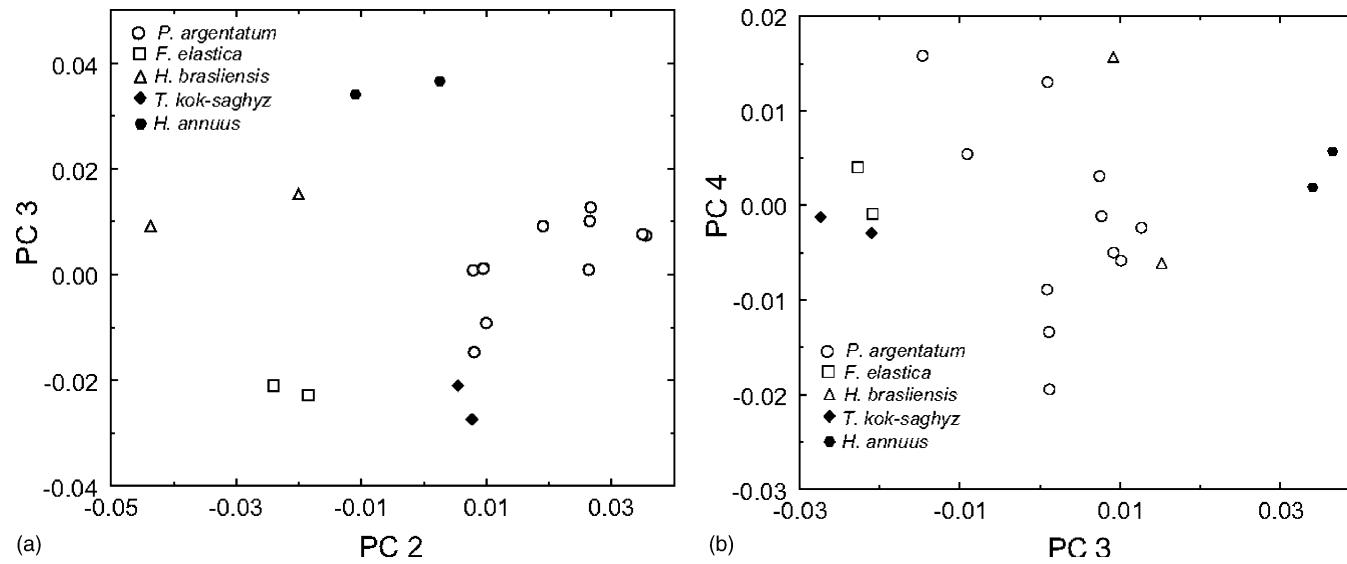


Fig. 9. Results of PCA analysis of rubbers from different species: (a) PC3 vs. PC2 and (b) PC4 vs. PC3.

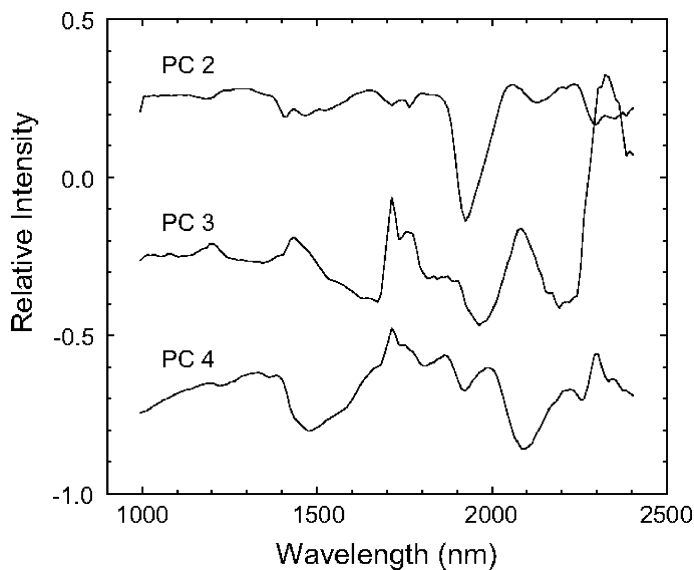


Fig. 10. Chemical features (loadings) that differentiate the rubbers from different species.

dominating the generalized model, five to eight samples from each of the four plant species, *F. elastica*, *H. annuus*, *H. brasiliensis* and *P. argentatum* were combined into one data set. One-half of the samples were used to construct a single, generalized PLS CALB model. This generalized CALB model was then used

to predict the natural rubber concentration of the other one-half of the samples (TEST set) (Fig. 11). As expected, the quality of the generalized model, as measured by its correlation coefficient or RMSEP, was not as high as that of the models constructed with only *P. argentatum* samples (Figs. 4 and 5). This is because

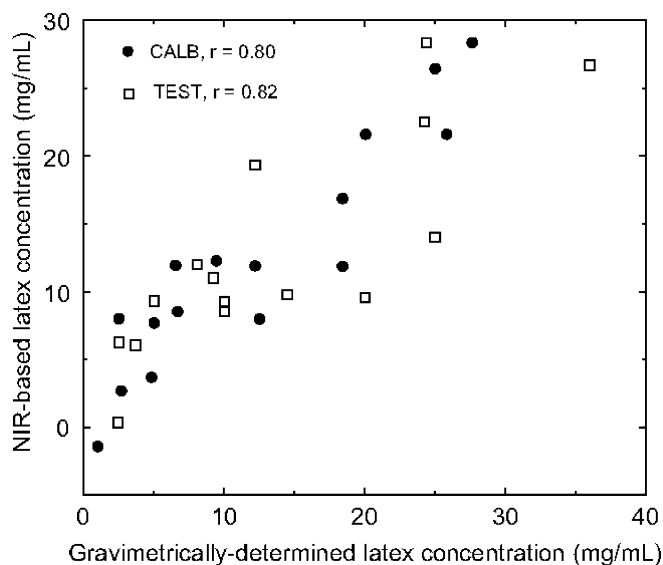


Fig. 11. Measured rubber concentration and rubber concentration predicted from NIR spectra of dry samples, including samples from all five species: (●) samples in the CALB set and (□) samples in the TEST set.

Table 2

Properties of PLS models constructed for rubber samples from different species

Rubber source	Correlation coefficient	RMSEP	Number of samples in model
<i>F. elastica</i> (latex)	0.94	4.8	7
<i>H. brasiliensis</i> (latex)	0.95	3.7	8
<i>H. annuus</i> (latex)	0.81	5.6	5

These models were constructed using full cross-validation, but without a separate TEST set. The concentration range was 0 and 35 mg/ml and two latent variables were used.

the rubber samples in the generalized model are from different species, and they differ in their proteins and resinous components, as well as in their natural rubber concentration.

However, the correlation coefficients and RMSEPs for the rubber content of each individual plant species (Table 2) are promising and show the potential for creating a generalized model or species-specific models to measure the rubber concentration in a wide variety of species.

The regression coefficients for the generalized PLS model (Fig. 6e) are very similar to the regression coefficients for the *P. argentatum* only model (Fig. 6a). The regression coefficients for the generalized model include strong features at 1710–1720 and 2290–2300 nm. They also include features at 1420 and 2100 nm. The peak at 1420 nm can be assigned to the natural rubber component, although the origin of the peak at 2100 nm is not clear (Black et al., 1985; Kleine and Foster, 1990; Marinho and Monteiro, 2000; Guilment and Bokobza, 2001).

4. Conclusions

The combination of NIR spectroscopy and PLS modeling can be used to measure the rubber concentration rapidly and accurately in both homogenate and latex samples from *P. argentatum*. These measurements can be conducted for either wet or dry samples and can be performed/run more rapidly than other quantification techniques. The NIR spectra also contain information on subtle chemical differences between the homogenate and latex samples. This NIR-based technique also appears promising for

measuring the rubber concentration in other plants such as *F. elastica*, *H. annuus*, *H. brasiliensis*, and *T. kok-saghyz*.

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