Communication

Determination of Ethanol in Gel Hand Sanitizers Using Mid and Near Infrared Spectroscopy

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Alcohol-based gel hand sanitizers became very popular during the COVID-19 (coronavirus disease 2019) pandemic. In Brazil, several irregular factories emerged requiring an efficient control by the police and regulatory agencies to guarantee product quality. This problem required a method to determine ethanol content, which led to the development of two methods employing mid and near infrared spectroscopy associated with chemometrics. Partial least squares (PLS) models were built and presented satisfactory results with mean absolute percentage error of prediction and root mean square error of prediction (RMSEP) of 1.12 and 0.76% (m/m), respectively, for mid-infrared (MIR) and 1.83 and 1.18% (m/m) for near-infrared (NIR). The analysis of commercial and seized samples of hand sanitizers showed that only 7 out of 34 samples had an ethanol content of 70% (m/m) or higher. This result reinforces the need for constant vigilance by authorities to ensure that the products have the required specifications.

Keywords: ethanol, hand sanitizers, infrared, PLS

Introduction

Due to the COVID-19 (coronavirus disease 2019) pandemic, the World Health Organization (WHO) has advised the public to clean their hands frequently to avoid contamination, causing alcohol-based gel hand sanitizers to become very popular. This has provoked a shortage of these products on the market. In order to counteract this, regulatory agencies worldwide such as the U.S. Food and Drug Administration (FDA) and the Brazilian Health Regulatory Agency (ANVISA) have issued guidelines for temporary preparation of these sanitizers by specific companies and pharmacies during this public health emergency in order to increase supply.[1,2]

In Brazil, ANVISA has also authorized the use of different feedstock than the ones recommended in the Brazilian Pharmacopoeia National Form. According to the Form, alcohol-based gel hand sanitizers should contain water, ethanol, carbomer 980 and triethanolamine.[3] Carbomer 980 is the component responsible for the gel consistency of a hand sanitizer and can be substituted by other carbomers or cellulose derived components, such as hydroxypropyl methylcellulose (HPMC) and hydroxyethyl cellulose (HEC). Triethanolamine is a neutralizing agent used to adjust pH value to obtain gel consistency when using carbormers. It can be substituted by other compounds such as aminomethyl propanol (AMP) or other bases. Additional components such as glycerol may be added for skin care. Also, ANVISA requires the ethanol content to be at least 70% (m/m) and that the product must have proven antibacterial activity.[4]

Although necessary, this temporary dismissal of product registration has caused irregular producers to emerge, disregarding proper manufacturing practices

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and often producing hand sanitizers that do not meet the specifications required for destruction of the virus, which gives the users a false sense of protection. Therefore, constant vigilance by the authorities is required in order to avoid the commercialization of subpar alcohol-based hand sanitizers.

Law enforcement agencies, who are responsible for the apprehension of irregular products, require a reliable and practical method to determine the ethanol content in gel hand sanitizers in order to evaluate if the amount of ethanol matches the value indicated on the product label. Some methods based on gas chromatography (GC), specific gravity using alcoholmeter, hydrometer or pycnometer and spectrophotometry have already been proposed for the analysis of ethanol solutions.[2] The problem with these methods is that the gel's viscosity hinders the usage of alcoholmeters and the other methods require specific equipment or destructive and time-consuming sample treatments. Mid-infrared (MIR) and near-infrared (NIR) spectroscopies associated with chemometrics are already being used to determine alcohol content in different kinds of samples such as beverages, fuels and fermentation broths.[5-10]

In this work, two methods were developed, both employing infrared spectroscopy, due to the fast and non-destructive nature of this technique and the small volume of sample required. One is based on attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and the other based on a handheld NIR spectrometer (MicroNIR). Several law enforcement laboratories already have FTIR spectrometers or could purchase a portable near-infrared device to be used for on-site measurements. These methods could also be used by the industry for quality control purposes.

Experimental

Samples

As the major components of alcohol-based hand sanitizers are ethanol and water, 13 samples of aqueous solutions of ethanol with concentrations ranging from 30 to 90% (m/m) were prepared with 99.8% ethanol (QEEL, São Paulo, Brazil) as well as 11 samples of gel hand sanitizers with ethanol concentrations ranging from 30 to 80% (m/m). Out of these 11 samples, 9 were prepared at the school pharmacy of the Federal University of Pernambuco (FECDA/UFPE). These contained anhydrous ethanol (donated by Pernambuco's ethanol plants), purified water, 0.5% of carbomer 940 (Dinâmica, Indaiatuba, Brazil) and AMP (Fagron, São Paulo, Brazil) in a sufficient

amount to obtain a pH between 6 and 7, according to the instructions described in the Brazilian Pharmacopoeia National Form.[3] As ANVISA[1] has also authorized the use of different feedstock than the ones already described in the Brazilian Pharmacopoeia National Form, two samples were prepared by the Pharmaceutical Laboratory of Pernambuco (LAFEPE) using anhydrous ethanol (Transálcool, Olinda, Brazil), purified water, HPMC (Denver Especialidades Químicas, Cotia, Brazil), and glycerol (Codossal Química, Recife, Brazil). Two of these 11 gel samples were used to compose the calibration set and the remaining were employed in the external validation set, as described in the Results and Discussion section.

In addition, 27 samples of commercial gel hand sanitizers were acquired in Recife plus 7 samples taken from seizures by the Pernambuco State Civil Police.

Data acquisition

A FTIR spectrometer (Spectrum Two, PerkinElmer) was employed for the acquisition of MIR spectral data. The spectra were acquired in the 650-4000 cm^{-1} range by averaging 4 scans with absorbance measurements every 1 cm^{-1} and spectral resolution of 4 cm^{-1}. The data were acquired using the ATR (attenuated total reflectance) accessory.

A handheld NIR spectrometer (MicroNIR™ Pro 1700, Viavi Solutions) was employed for the acquisition of NIR spectral data. The spectra were acquired in the 908-1676 nm range by averaging 100 scans with an integration time of 12.5 ms and with absorbance measurements every 6 nm, approximately. The data were acquired in the transmittance mode using a laboratory-made accessory, based on the one described in Paiva *et al*.[11] Quartz cuvettes with 2 and 5 mm optical path lengths were employed in order to evaluate the best option for spectra acquisition.

All spectra acquisitions were performed in triplicate and used as different samples for the calculations.

Data analysis

Several pre-processing techniques were evaluated, such as standard normal variate (SNV), multiplicative scatter correction (MSC) and 1st and 2nd derivative with a Savitzky-Golay filter (2nd order polynomial with a 15-point window for MIR data and a 7-point window for NIR data). Before pre-processing, the spectral range from 650-790 cm^{-1} of the MIR spectra was excluded because it contained high noise. The best pre-processing technique was chosen based on the best results obtained in the partial least squares (PLS) regression models.

Three types of models were built: (*i*) models using only the samples of aqueous solutions of ethanol; (*ii*) models using all samples of aqueous solutions of ethanol plus 2 samples of gel hand sanitizers; and (*iii*) models with all samples of aqueous solutions of ethanol and 4 samples of gel hand sanitizers. The remaining 7 ethanol-based gel hand sanitizers samples were used to evaluate the prediction ability of the models. Univariate models were also built in order to compare the results. All data analyses were performed using PLS_Toolbox 8 (Eigenvector Research, Inc) in the MATLAB® (MathWorks) environment.[12]

Results and Discussion

In the MIR region (Figure 1a), the characteristic bands of ethanol and water are related to O–H intermolecular hydrogen bond stretching (3400-3200 cm⁻¹), C–H stretching (2980 and 2885 cm⁻¹), H_2O bending (1640 cm⁻¹), C–H bending (1380 and 1455 cm⁻¹) and C–O stretching (1085, 1045 and 880 cm⁻¹).[13]

The NIR spectra acquired with the 5 mm path length cuvette showed saturated absorption signals, particularly in the region between 1400 and 1500 nm. Based on this, the 2 mm optical path length cuvette was selected for the measurements performed in this work. In the NIR region (Figure 1b), the characteristic bands of ethanol and water are the combination band involving the stretching modes of the water molecule (1460 nm) and the O–H combination band (1580 nm).[14]

Gel hand sanitizers samples were used in the calibration set to increase variability in the PLS models, as the gel sanitizers have a more complex composition than aqueous solutions of ethanol. The addition of 2 gel samples (50 and 70% ethanol content) improved prediction results, with lower values of root mean square error of prediction (RMSEP), mean absolute percentage error (MAPE) and bias when compared to the models without gel samples. However, the addition of 4 gel samples (50, 70, 70 and 80% ethanol content) did not significantly increase the prediction ability of the model when compared to the models with just 2 gel samples. Therefore, the models with 2 gel samples were selected as the best and they were used to perform the prediction of the remaining 9 gel samples. Univariate models were also built using the variables with the highest variable importance in projection (VIP) scores of the models with 2 gel samples in the calibration set and the best pre-processing techniques. The analytical curves for the univariate models are presented in Supplementary Information (SI) section. The NIR data with MSC pre-processing did not produce a linear univariate model and therefore the 2ⁿᵈ derivative was used. Although the values at only one wavelength were employed to build the univariate models, the spectra (or at least a spectral range) have to be acquired in order to pre-process the data. Only the results with the best pre-processing techniques are presented in Table 1.

The PLS type 2 NIR model presented significantly different values (according to *F*-test at a 95% confidence level) of root mean square error of cross validation (RMSECV) and RMSEP than the MIR model. Higher values of MAPE of prediction were also observed for the NIR model. These results may be attributed to the use of a handheld spectrometer with a limited spectral range. On the other hand, the handheld spectrometer is a cheaper instrument and can be used on the field.
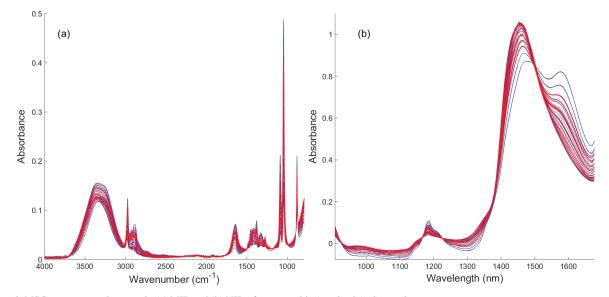

Figure 1. MSC pre-processed spectra in (a) MIR and (b) NIR of aqueous (blue) and gel (red) samples.

Table 1. Results for the PLS regression and univariate models

	Model	Pre-processing	LVs	RMSECV / (% m/m)	RMSEP / (% m/m)	Bias of prediction	R^2 of prediction	MAPE of prediction / %
	type 1	MSC	4	0.44	1.28	−0.98	0.9983	1.98
MIR	type 2	MSC	3	0.51	0.76	−0.07	0.9984	1.12
	univariate (1046 cm^{-1})	MSC			1.61	0.90	0.9939	2.64
	type 1	2nd derivative	4	0.77	1.38	0.50	0.9962	2.34
NIR	type 2	MSC	4	0.68	1.18	−0.28	0.9950	1.83
	univariate (1378.87 nm)	2nd derivative			1.21	0.71	0.9967	2.08

Type 1 model uses only the samples of aqueous solutions of ethanol; type 2 model uses all samples of aqueous solutions of ethanol plus 2 samples of gel hand sanitizers. LVs: latent variables; RMSECV and RMSEP: root mean square error of cross validation and prediction, respectively; R^2: coefficient of determination; MAPE of prediction: mean absolute percentage error of prediction; MIR: mid-infrared; NIR: near-infrared; MSC: multiplicative scatter correction.

Figure 2 shows the predicted *versus* reference plots for the prediction set (9 gel samples) using PLS (type 2) and univariate models.

Even though the values of RMSEP, MAPE of prediction and bias of the univariate models were higher than the values observed for the multivariate models, the univariate models can still be used if necessary, as long as the variable is obtained from the preprocessed data.

Finally, the commercial and seized samples had their ethanol content determined using the best PLS models. The results (presented in SI section) showed that only 7 samples out of the 34 had an ethanol content of 70% (m/m)
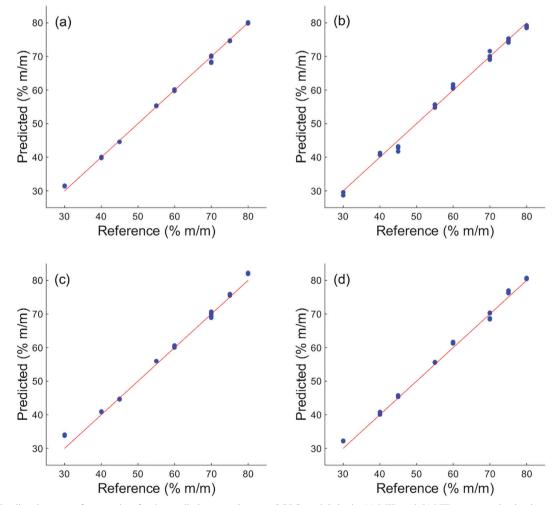

Figure 2. Predicted *versus* reference plots for the prediction set using type 2 PLS models in the (a) MIR and (b) NIR ranges and univariate models in the (c) MIR and (d) NIR ranges. The line shown is the quadrant bisectrix.

or higher considering the 95% confidence interval. The average concentration of these samples' triplicates varied from 23.5 to 75.6% (m/m) and from 20.3 to 77.2% (m/m) using MIR and NIR type 2 models, respectively.

Conclusions

In this work, simple, fast and non-destructive methods based on MIR and NIR spectra were developed to determine the ethanol content in alcohol-based gel hand sanitizers. These methods are important for the police and regulatory agencies to ensure product quality. They can also be used in industries for quality control purposes. The results demonstrated that the addition of 2 gel samples to the calibration set improved prediction errors and bias compared to models with only aqueous solutions of ethanol and water. Ethanol content in commercial and seized samples was determined using the best PLS models demonstrating that only 7 samples out of the 34 had an ethanol content of 70% (m/m) or higher. This result reinforces the need for constant vigilance by the authorities to ensure that the products have the required specifications.

Supplementary Information

Supplementary information (Figure S1, Table S1) is available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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