Journal of Pharmaceutical and Biomedical Analysis 66 (2012) 252–257

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Near infrared spectroscopy and multivariate calibration for simultaneous determination of glucose, triglycerides and high-density lipoprotein in animal plasma

Ana Carolina de Oliveira Neves[a], Aurigena Antunes de Araújo[b], Bruna Laís Silva[b], Patrícia Valderrama[c], Paulo Henrique Março[c], Kássio Michell Gomes de Lima[a,*]

[a] Universidade Federal do Rio Grande do Norte, Instituto de Química, Grupo de Pesquisa em Quimiometria Aplicada, CEP 59072-970 Natal, RN, Brazil
[b] Department of Biophysics and Pharmacology, UFRN, 59072-970 Natal, Brazil
[c] Universidade Tecnológica Federal do Paraná, Coordenação de Licenciatura em Química, BR 369, Km 0.5, CEP 87301-006 Campo Mourão, PR, Brazil

ARTICLE INFO

Article history:
Received 23 December 2011
Received in revised form 13 March 2012
Accepted 13 March 2012
Available online 23 March 2012

Keywords:
NIR
Glucose
Triglycerides
High-density lipoprotein
Multivariate calibration

ABSTRACT

The quantitative analysis of glucose, triglycerides and high-density lipoprotein (HDL) in rat plasma without sample pre-treatment using direct near-infrared spectroscopy was studied. Comparison was made of several multivariate calibration techniques and algorithms for data pre-processing and variable selection, including partial least squares (PLS), interval partial least squares (iPLS), genetic algorithm (GA) and successive projections algorithm (SPA). Variable selection yielded good results for the correlation coefficient and Root Mean Square Error of Prediction (RMSEP) values for the three parameters, especially triglycerides. The RMSEP values for glucose, triglycerides and HDL produced by the PLS model were 6.08, 16.07 and 2.03 mg dl^{-1}, respectively. *F* tests and *t*-tests were performed to compare the results of the models with each other and with a reference method. These results suggests that the PLS method can be used to simultaneously determine the concentrations of glucose, triglycerides and HDL in complicated biological fluids with NIR spectroscopy, offering an alternative analysis in animals.

1. Introduction

The values of hematological parameters in rats used in experiments are influenced by the housing and environment around the animal. Although each rat species has mechanisms to maintain proper values of these parameters, variations arise from sex, strain and genotype as well as age, diet, handling of the animals and the surrounding environment. Further, animals subjected to an experiment may respond differently depending on their environmental conditions and may be influenced by various ecological factors that vary by geographical region [1].

For biochemical laboratory measurements, quantitative methods must be suitably accurate and precise over the expected range of values required. In the literature, the vast majority of the biochemical analyses of rat hematological parameters, including glucose levels, triglycerides and cholesterol [2–5], are made by enzymatic methods. Plasma glucose levels are determined in animals that have fasted for 12 h using a colorimetric enzymatic assay with glucose oxidase. Triglycerides are determined using a colorimetric enzymatic assay with glycerol-3-phosphate oxidase, in which lipases act on triglycerides to generate glycerol, which is subsequently converted into glycerol phosphate and then oxidized to water and hydrogen peroxide by glycerol-3-phosphate oxidase. Hydrogen peroxide then reacts with 4-aminoantipyrine and 4-chlorophenol through an oxidative reaction catalyzed by peroxidase, producing a quinone imine (red) with a color intensity that is proportional to the original concentration of triglycerides in the sample. Cholesterol (HDL), in turn, is determined by a colorimetric enzymatic assay with cholesterol oxidase. In this method, cholesterol oxidase catalyzes the oxidation of cholesterol to produce hydrogen peroxide, which is then converted to red-colored (quinine imine) as described above where the color intensity is proportional to the original concentration of cholesterol in the sample. However, all of these methods for determining glucose, triglycerides and HDL in the plasma of animals suffer from inherent difficulties that affect the speed of analysis and the results. These problems include the need for specific reagents and temperature control, problems of chemical interference and the time required for analysis.

NIR spectroscopy has been applied successfully for the qualitative and quantitative analysis of several classes of compounds in areas such as the pharmaceutical industry [6], food safety [7], environmental studies [8], and clinical practice [9], among others. In the analysis of biochemical compounds, its advantages

* Corresponding author. Tel.: +55 84 3215 3828; fax: +55 83 3211 9224.
E-mail address: kassio@ufrnet.br (K.M.G. de Lima).

0731-7085/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2012.03.023

include an absence of specific reagents and non-destructive and non-invasive measurements. Various types of biomedical samples, including glucose [10,11], human serum albumin [12], cholesterol [13], urea [14] and hematocrit [15], have been investigated by NIR spectroscopy.

Hazen et al. [10] determined the total protein, albumin protein, globulin protein, triglycerides, cholesterol, urea, glucose and lactate plasma levels using NIR spectroscopy and multivariate calibration. PLS models gave RMSEP values of 3.23 mg dl^{-1} for glucose. Kang et al. [16] employed NIR spectroscopy and multivariate tools for the quantification of cholesterol, glucose and urea in bovine samples. The best predicted results for cholesterol, glucose and urea had a RMSEP of 6.68, 10:35 and 1.28 mg dl^{-1}, respectively. Kasemsumran et al. [17] developed a PLS models using NIR spectroscopy for the determination of glucose in cattle blood serum samples. The authors obtained models showing values of the RMSEP of 25.31 mg dl^{-1} and a correlation coefficient of 0.99 with eight latent variables. In another experiment, Petter et al. [18] proposed to determinate the LDL and high density lipoprotein (HDL) content in human serum by employing near-infrared (NIR) spectroscopy and multivariate calibration techniques (PCR and PLS). The authors used as an adsorbent (TiO(2) beads) for selectively immobilizing LDL and HDL-cholesterol and further analyzing the incubated and washed samples via NIR diffuse reflection spectroscopy. In this work, the pretreated serum samples were predicted by the PLSR model while the standard deviation (SD) from the reference to the NIR predicted values for six test samples in a concentration range from 500 to 2500 ppm were lower than 10%. Finally, Filho and Poppi [19] studied the use of NIR spectroscopy and multivariate calibration methods to measure triglyceride levels in human plasma. The authors compared the performance of techniques such as PLS, MLR and genetic algorithms as methods for variable selection. For the measurement of triglyceride levels, the methodology showed errors of approximately 9%, which is an acceptable relative error for this parameter.

However, several complicating factors remain. The major difficulties in the analysis of blood components by NIR are the weakness of the NIR signals from blood components compared to those from other components, especially water, and the complexity of overlapping bands. To overcome these difficulties, various chemometric algorithms have been applied to NIR data from serum, whole blood, and human tissues. The methods used for variable selection, such as iPLS (interval partial least squares) [20], GA (genetic algorithm) [21] and SPA (successive projections algorithm) [22], allow for improved multivariate models using a spectrum of variables with more relevant information. These algorithms eliminate variables that do not directly correlate with the property of interest, such as those that add only noise, nonlinearities, or irrelevant information. They also eliminate potential interferences and variables that generate a lower signal/noise ratio (which is indicative of low sensitivity).

Another tool used to improve NIR results is outlier detection, which selects samples that depart from the bulk of the data due to instrumental errors, the presence of another population, laboratory errors, and so on. The calibration and prediction sets in this work were optimized based on data with extreme leverage, unmodeled residuals in spectral data, and unmodeled residuals in the dependent variables [23].

In this work, a quantitative analysis of glucose, triglycerides and HDL in rat plasma was performed without sample pre-treatment, using direct NIR absorption measurements. A comparison of several multivariate calibration techniques, including PLS, iPLS, SPA, GA and outlier detection, was performed to determine the best models. Additionally, several data pre-processing methods were compared to determine which method was best to analyze this kind of data. These methods will be useful as an alternative to the existing methods for the simultaneous monitoring of glucose, triglycerides and HDL in plasma.Experimental

2.1. Animals

Twenty-three adult male Labina rats, weighing 300 g on average, were provided by the Animal Department of Biophysics and Pharmacology, Federal University of Rio Grande do Norte. All the animals were exposed to the same environmental conditions: light controlled in a 12/12 h light/dark cycle, temperature, and food and water ad libitum. The food of the animals was removed to induce fasting for 12 h before sacrifice. This study used two groups of rats, designated as control and experimental groups, with four subgroups in each group. The control group received saline injected intraperitoneally (ip), while the experimental group received a single dose of 4 mg kg^{-1} tobramycin ip. The data used in this study were obtained from a pre-clinical experimental study performed on rats which aimed the evaluation of the biochemical parameters (Glucose, HDL and triglycerides) during the use of tobramycin (antibiotic) over a period of 04 weeks. This study attempted to simulate the conditions of the dose quantity and duration of antibiotic therapy with tobramycin in humans, since it is used for the antibiotic treatment of serious infections potentially caused by *Pseudomonas aeruginosa* [24,25]. Each week, a subset of each group was euthanized with sodium thiopental at a dosage of 50 mg kg^{-1}, according to a protocol approved by the Ethics Committee on Animal Use, and blood was taken for the biochemical evaluation of glucose, triglyceride and HDL levels.

2.2. Reference method

Blood samples from sacrificed animals were collected in 15 ml conical Falcon tubes (16.5 mm × 120 mm) and left at room temperature (25 °C) to clot. Serum was obtained by centrifugation at 3000 rpm for 10 min. Blood glucose levels were measured using a previously described glucose oxidase method [1], using an analytical kit supplied by Bioclin, Brazil and following the instructions provided. The absorbance of the samples was determined using a BioPlus 2000. HDL cholesterol esters were measured using an analytical assay kit supplied by Bioclin, Brazil, following the instructions provided. Triglycerides were measured by the glycerol kinase method, using a kit supplied by Bioclin, Brazil and following the instructions provided. The concentration ranges of glucose, triglycerides and HDL were 73–130, 22–135 and 25.4–52.7 mg dl^{-1}, respectively. The estimated standard deviations for these measurements in rat plasma were 17.4, 24.7 and 11.4 mg dl^{-1}, respectively [1]. The study was approved by the Ethics and Research 36, UFRN.

2.3. Instrumentation

Spectral measurements were performed using a MB 160 Bomem FT-NIR spectrophotometer (ABB Bomem, Quebec, Canada). NIR spectra were obtained over a range of 1100–2500 nm with a resolution of 8 cm^{-1}. The time measurement was 41 s (50 scans) per spectrum. Absorbance spectra of rat plasma were obtained against an empty quartz cell (1 mm optical path, NSG precision cells, Inc., model 21UV1). The sample was introduced into a quartz cell using a disposable 1 ml syringe. After each measurement, the cell was cleaned using a sequence of 1 M acetic acid, ultra-pure water and finally acetone to dry the cell. Temperature was kept at 25 °C through the experiments.Data analysis

Data analysis was performed using MATLAB version 6.5 (The Math-Works, Natick, USA) using the PLS-toolbox (Eigenvector Research, Inc., Wenatchee, WA, USA, version 6.01). Different preprocessing methods were used, including the derivative and smoothing Savitzky–Golay methods, with varying the number of

window points (3, 5 and 7). The samples were divided into calibration (17 samples) and prediction (6 samples) sets by applying the classic Kennard-Stone (KS) selection algorithm [26] to the NIR spectra. The lowest root mean square error of prediction (RMSEP) is obtained when using the optimum number of PLS factors, which is found using the variance of the matrix of the instrumental responses. The prediction set was used to test the predictive ability of the PLS models. The predicted results for the calibration models developed by PLS using the spectral regions selected by iPLS, GA, and SPA were compared to those found by PLS using the whole region. Finally, the best models results for each parameter were compared before and after applying outlier detection.

3. Results and discussion

The organic matrix of this study, serum was obtained from animals receiving tobramycin in order to simulate the conditions of treatment period (04 weeks) held in humans. As this pharmacological, tobramycin, has the adverse effect is renal toxicity. The toxicity results from accumulation and retention of aminoglycoside (tobramycin) in the renal proximal tubular cells [27,28] and the levels of glucose, HDL and triglycerides were measured each week.

The absorbance spectra of 23 blood plasma samples of rats were directly measured with single-beam spectra. A representative spectrum is given in Fig. 1. The strong absorption band near to 1450 nm was assigned to the combination of OH symmetric and antisymmetric stretching modes, and a saturated feature of approximately 1940 nm was assigned to the combination of OH stretching and bending vibrations. For the clinical analyses, the presence of water is problematic because it shows a strong absorption in the same range as biological species such as glucose, triglycerides and cholesterol. Because of this, it is necessary to pre-process the data to decrease or remove water absorption effects. Here, to avoid the strong absorption of water, the region approximately at 1900–2000 nm was eliminated before the development of the calibration models for analytes (glucose, triglycerides and HDL). However there are informative NIR regions of glucose, triglycerides and cholesterol between 1100–1900 nm and 2000–2500 nm [13] for multivariate models. The bands arising from combinations of the stretching and deformation modes of CH and NH groups are found in this region, making it suitable for collecting useful information about the three analytes and for establishing PLS models.

3.1. Glucose

The results obtained for the calibration models in the NIR region for glucose in rat plasma are shown in Table 1. In addition to the PLS models, the results of the PLS–SPA, PLS-GA and iPLS models are shown. Only the best results from the tested pre-processing techniques are presented. *F* tests were performed for all the models using the prediction set. The results showed no significant difference (95% confidence level) between the best PLS model, the PLS (4)[1] and the other models, except for the PLS (4) first derivative (3 pts), the PLS (3) first derivative (7 pts), the PLS (3) second derivative (5 pts), the iPLS (3) using 400 spectral variables and the iPLS (4) using 600 spectral variables. Therefore, variable selection was important in this work because it enabled the use of models that require a small number of spectral variables. The RMSEP values of all the models were less than the reproducibility value of the BIOCLIN (Brazil) reference method, except for the PLS (4) first derivative (3 pts). The correlation coefficients for the prediction set ranged from 0.17 to 0.97 for all the models. It was observed that, in the NIR spectral region, models with derivative data showed higher RMSEP values than models with raw or smoothed data. Some important spectral information may have been lost when the derivative spectra were employed. The number of latent variables used for the PLS, iPLS, SPA and GA models using NIR spectra was 3 or 4. With the PLS models, a narrow spectral region (1105.05–1892.3 nm and 1997.27–2461.97 nm) was enough to predict the glucose parameter. The strategy of using GA models had the advantage of using few variables (321) to build the PLS models.

Two outliers were excluded from the calibration set, and the best PLS model for glucose was developed by applying a smoothing with 3 pts. For this model, the lowest Root Mean Square Error of Cross Validation (RMSECV) and RMSEP were 12.10 and 6.08, respectively. The correlation coefficient for the validation set was 0.98 and was obtained using four latent variables. This model was not significantly different when compared with the reference values according to a *t*-test (95% confidence level). Fig. 2 shows that the correlation between the measured and predicted values for glucose in rat plasma was predicted from NIR spectroscopy with error values similar to that of the reference method (17.4 mg dl^{-1}) [1].

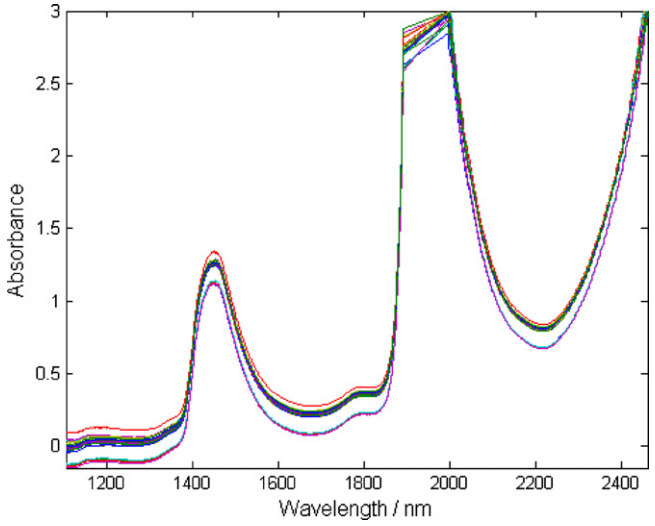

Fig. 1. NIR spectra of 23 samples of blood plasma of rats.

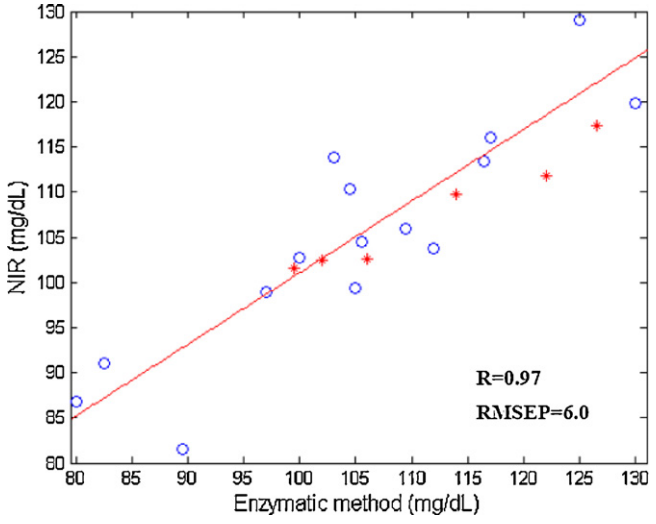

Fig. 2. Correlation among the measured and the predicted values for both calibration and prediction sets for glucose in rat blood plasma for the best model, PLS (4)[1], by NIR spectroscopy. (○) calibration set; (✱) validation set.

Table 1
Results for calibration and the external validation set for glucose: root mean square error of cross validation (RMSECV) and prediction (RMSEP), correlation coefficient (*R*) and the number of used spectral variables (Size). The number of PLS factors or in PLS, iPLS, PLS-SPA and PLS-GA models are represented in parentheses.

Models[a]	Calibration		Prediction		Size
	R	RMSECV (mg dl^{-1})	*R*	RMSEP (mg dl^{-1})	
PLS (4)	0.87	13.17	0.85	8.01	1223
PLS (4), smoothing 3 pts	0.84	13.46	0.93	6.32	1221
PLS (4), smoothing 5 pts	0.80	14.78	0.96	5.50	1219
PLS (4) First deriv. 3 pts	0.92	20.36	0.17	19.47	1221
PLS (4) First deriv. 5 pts	0.87	21.36	0.36	14.21	1219
PLS (3) First deriv. 7 pts	0.80	15.67	0.20	14.77	1217
PLS (3) Sec. deriv. 5 pts	0.88	26.00	0.83	12.14	1219
iPLS (3)	0.99	13.80	0.24	12.49	122
iPLS (3)	0.82	14.06	0.30	16.31	400
iPLS (4)	0.87	13.26	0.47	16.13	600
PLS-SPA (3)	0.80	13.46	0.11	12.02	17
PLS-GA (4)	0.90	9.63	0.84	10.80	321
PLS (4)[1]	0.90	12.11	0.97	6.08	1221

[a] Pts, points; deriv., derivative; sec., second.; [1] one application of outliers detection.

3.2. Triglycerides

Table 2 shows the results for the analysis of triglycerides in rat plasma. In general, better values were obtained for the RMSEP with smoothed data (3 pts), when compared with the models obtained with original raw or preprocessed data. For the suitable NIR spectral region (1105.05–1892.3 nm and 1997.27–2461.97 nm) the RMSEP values obtained for all of the models were similar, except for the PLS (3) first derivative (3 pts), PLS (1) first derivative (7 pts) and PLS (2) second derivative (5 pts) models. An *F*-test was performed for all of the PLS models using the prediction set, and the results showed no significant differences at the 95% confidence level among the best model, the PLS (3) smoothing (3 pts) model, and the other models, except for the three models already mentioned that presented the highest RMSEP values (30.80–37.30 mg dl^{-1}). For this parameter, variable selection using the iPLS, SPA and GA algorithms produced good results. For example, according to Table 2, for the PLS-SPA (3) model, a correlation coefficient of 0.91 was obtained for the prediction set using only seventeen spectral variables, and a RMSEP was obtained with a value under of the reproducibility value of the BIOCLIN (Brazil) reference method (24.7 mg dl^{-1}) [1]. The best variable selection algorithm for this parameter was found to be the iPLS algorithm. When 122 spectral variables were used to build the iPLS (3) model, a correlation coefficient for the prediction set of 0.96 was found; this value was close to that obtained for the best model (0.96). This model presented a higher RMSEP value, though it was still below that of the reference error.

Outlier detection was applied to the best model, but no improvement of the results was observed. To build the PLS (3)[1] model, three samples of the calibration set were excluded. After this selection, the same PLS (3)[1] was submitted to a second outlier detection, resulting in one outlier identified in the calibration set and another outlier in the prediction set. These two samples were excluded, and a new model PLS (3)[2] was built.

Fig. 3 shows the correlation between the measured values for triglycerides in rat plasma and those predicted by the best model, PLS (3) smoothing (3 pts), based on NIR spectroscopy. A *t*-test was performed and showed a significant difference (95% confidence level) between this model and the reference method. Because this result was not satisfactory, the *t*-test was applied at a 96% confidence level, resulting in no significant difference when compared to the reference method. The model provided a RMSEP value that was similar to the one observed by Filho et al. (20 mg dl^{-1}) [19].

3.3. Cholesterol

Table 3 shows the results for the cholesterol models. In general, the best values were obtained for the RMSEP with derivative data (3 pts), instead of original raw data or preprocessed data, in the spectral region from 1105.05 to 1892.3 nm and 1997.27 to 2461.97 nm. For all of the fourteen models presented in Table 3, the RMSEP values were below the reproducibility value of the BIOCLIN (Brazil) reference method (11.4 mg dl^{-1}). However, the correlation coefficients for the prediction set ranged from −0.62

Table 2
Results for calibration and the external validation set for triglycerides: root mean square error of cross validation (RMSECV) and prediction (RMSEP), correlation coefficient (*R*) and the number of used spectral variables (Size). The number of PLS factors or in PLS, iPLS, PLS-SPA and PLS-GA models are represented in parentheses.

Models[a]	Calibration		Prediction		Size
	R	RMSECV (mg dl^{-1})	*R*	RMSEP (mg dl^{-1})	
PLS (3)	0.77	26.16	0.94	21.58	1223
PLS (3), smoothing 3 pts	0.80	25.75	0.96	16.07	1221
PLS (3), smoothing 5 pts	0.78	24.54	0.94	16.75	1219
PLS (3) First deriv. 3 pts	0.89	34.25	0.40	35.75	1221
PLS (3) First deriv. 5 pts	0.78	38.84	0.80	21.34	1219
PLS (1) First deriv. 7 pts	0.56	28.41	0.75	30.79	1217
PLS (2) Sec. deriv. 5 pts	0.61	32.19	0.41	37.29	1219
iPLS (3)	0.75	27.03	0.96	21.19	122
iPLS (3)	0.76	25.90	0.93	26.00	400
iPLS (4)	0.74	25.74	0.94	24.83	600
PLS-SPA (3)	0.80	28.83	0.91	20.62	17
PLS-GA (3)	0.73	25.62	0.90	20.64	357
PLS (3)[1]	0.81	24.18	0.91	20.91	1221
PLS (3)[2]	0.83	23.06	0.93	15.76	1221

[a] Pts, points; deriv., derivative; sec., second.; [1] one application of outliers detection; [2] two applications of outliers detection.

Table 3
Results for calibration and the external validation set for HDL: root mean square error of cross validation (RMSECV) and prediction (RMSEP), correlation coefficient (*R*) and the number of used spectral variables (Size). The number of PLS factors or in PLS, iPLS, PLS-SPA and PLS-GA models are represented in parentheses.

Models[a]	Calibration		Prediction		Size
	R	RMSECV (mg dl^{-1})	*R*	RMSEP (mg dl^{-1})	
PLS (4)	0.88	6.22	0.05	8.24	1223
PLS (4), smoothing 3 pts	0.87	5.91	0.03	8.60	1221
PLS (4), smoothing 5 pts	0.87	5.76	0.02	8.77	1219
PLS (4) First deriv. 3 pts	0.87	10.51	0.65	4.56	1221
PLS (2) First deriv. 5 pts	0.76	8.04	-0.35	7.64	1219
PLS (3) First deriv. 7 pts	0.80	9.62	-0.01	7.39	1217
PLS (2) Sec. deriv. 5 pts	0.64	8.92	0.64	4.63	1219
iPLS (3)	0.87	5.75	-0.62	8.00	122
iPLS (3)	0.75	7.51	0.06	7.43	400
iPLS (4)	0.72	7.92	0.24	6.83	600
PLS-SPA (4)	0.77	13.80	0.51	6.30	17
PLS-GA (4)	0.98	3.49	0.81	3.31	334
PLS (4)[1]	0.98	3.75	0.95	1.66	334
PLS (4)[2]	0.99	4.60	0.98	2.03	334

[a] Pts, points; deriv., derivative; sec., second.; [1] one application of outliers detection; [2] two applications of outliers detection.

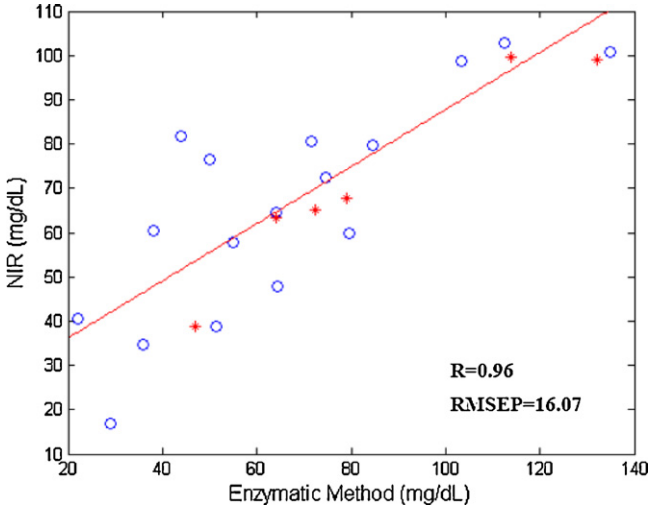

Fig. 3. Correlation among the measured and the predicted values for both calibration and prediction sets for triglycerides in rat blood plasma for the best model, PLS (3) smoothing 3 pts, by NIR spectroscopy. (○) calibration set; (✱) validation set.

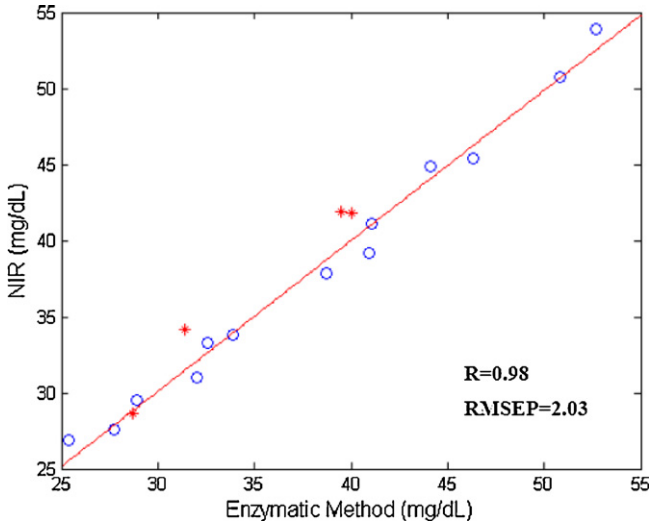

Fig. 4. Correlation among the measured and the predicted values for both calibration and prediction sets for HDL in rat blood plasma for the best model, PLS (4)^2, by NIR spectroscopy. (○) calibration set; (✱) validation set.

to 0.98, indicating that some models were unsatisfactory. For the specified NIR spectral region, the RMSEP values were similar for several models: the PLS (4) first derivative (3 pts), the PLS (2) second derivative (5 pts), the iPLS (4) using 600 spectral variables, the PLS-GA (4), the PLS (4)^1 after one application of outlier detection and the PLS (4)^2 after two applications of outlier detection. An *F* test was performed on these models using their prediction sets, and the results shows no significant differences (95% confidence level) among them. Another important issue for this parameter is variable selection. Using the GA method to choose the main spectral variables for use in the models, the correlation coefficient obtained for the prediction set was 0.81, and the RMSEP was 3.31 (334 spectral variables). This model was submitted to outlier detection, and two samples of the calibration set and one sample of the prediction set were excluded to give a new model, the PLS(4)^1. This latter model generated a correlation coefficient for the prediction set of 0.95 and a RMSEP of 1.66. Then, the PLS (4)^1 model was submitted to an additional round of outlier detection, and this excluded three more samples (two of the calibration set and one of the prediction set) in addition to the previous three. For the PLS (4)^2 model, (two more samples of the calibration set were excluded) the lowest RMSECV and RMSEP values were 4.60 and 2.03, respectively. The correlation coefficient for the validation set, obtained using 4 latent variables, was 0.98. Fig. 4 shows the correlation between the measured values of HDL in rat plasma and those predicted from NIR spectroscopy by the PLS (4)^2 model.

4. Conclusions

In this work, it was demonstrated that it is possible to obtain promising results considering the concentration range used in the calibration/validation sets (The RMSEP values for glucose, triglycerides and HDL found in this work were 6.08, 16.07 and 2.03 mg dl^{-1}, respectively) for the quantitative analyses of glucose, triglycerides and cholesterol in the blood plasma of rats with NIR spectroscopy and multivariate regression methods. The optimal combination of the informative regions from the iPLS, GA and SPA models directly improved the predicted values of triglycerides concentrations in blood plasma. For glucose and cholesterol, the models built using a GA method showed better results than those obtained using SPA or iPLS algorithms. For comparison, PLS models built by Petter el al. to HDL (in serum human samples by NIR) showed errors smaller than 10% [18]. In this work errors were found for HDL as being 4.9%. Furthermore, the relative errors found (PLS models) in this work for glucose and triglycerides were 4.1% and 13.4%, respectively. An important aspect of this approach is that

no reagents are necessary except for the aqueous solutions used to clean and rinse the optical cell. In addition to increasing the speed of the analysis, the proposed method may also provide a reduction in waste production compared to traditional methods.

Acknowledgements

The authors wish to thank the CAPES for a fellowship for A.C.O. Neves, the Programa de Pós-Graduação em Química (PPGQ) da UFRN and the Laboratório de Farmacologia da UFRN for providing the blood samples.

References

[1] J.A. Dantas, C.R. Ambiel, R.K.N. Cuman, S. Baroni, C.A.B. Amado, Valores de referência de alguns parâmetros fisiológicos de ratos do Biotério Central da Universidade Estadual de Maringá, Estado do Paraná, Acta Sci. Health Sci. 28 (2006) 165–170.
[2] M.R.V. Santos, V.H. Souza, I.A.C. Menezes, J.L. Bitencourt, J.M. Resende-Neto, A.S. Barreto, F.C. Andrade, R.M. Marçal, F. Teixeira-Silva, L.J. Quintans-Júnior, A.P.O. Barbosa, Parâmetros bioquímicos, fisiológicos e morfológicos de ratos (Rattus novergicus linhagem Wistar) produzidos pelo Biotério Central da Universidade Federal de Sergipe, Scientia Plena 6 (2010) 1–6.
[3] D.S.C.N. Pinheiro, C.B.F. Favali, A.A.S. Filho, P.V. Costa, Parâmetros Hematológicos de Camundongos e ratos do Biotério Central da Universidade Federal do Ceará, Bol. Inf. Cobea 3 (1997) 6–9.
[4] L.E. Lillie, N.J. Temple, L.Z. Florence, Reference values for young normal Sprague-Dawley rats: weight gain, hematology and clinical chemistry, Hum. Exp. Toxicol. 15 (1996) 612–616.
[5] S.T. Wolford, R.A. Schroer, F.X. Gohs, P.P. Gallo, M. Brodeck, H.B. Falk, R. Ruhren, Reference range database for serum chemistry and hematology values in laboratory animals, J. Toxicol. Environ. Health 18 (1986) 161–188.
[6] A.C.O. Neves, G.M. Soares, S.C. de Morais, F.S.L. da Costa, D.L. Porto, K.M.G. de Lima, Dissolution testing of isoniazid, rifampicin, pyrazinamide and ethambutol tablets using near-infrared spectroscopy (NIRS) and multivariate calibration, J. Pharm. Biomed. Anal. 57 (2012) 115–119.
[7] M.R.C. Inacio, M.F. Moura, K.M.G. de Lima, Classification and determination of total protein in milk powder using near infrared reflectance spectrometry and the successive projections algorithm for variable selection, Vib. Spectrosc. 57 (2011) 342–345.
[8] P. Geladi, H. Bärring, E. Dåbakk E, J. Trygg, H. Antti, S. Wold, B. Karlberg, Calibration transfers for predicting lake-water pH from near infrared spectra of lake sediments, J. Near Infrared Spectrosc. 7 (1999) 251–264.
[9] A. Sakudo, Y.H. Kato, H. Kuratsune, K. Ikuta, Non-invasive prediction of hematocrit levels by portable visible and near-infrared spectrophotometer, Clin. Chim. Acta 408 (2009) 123–127.
[10] K.H. Hazen, M.A. Arnold, G.W. Small, Measurement of glucose and other analytes in undiluted human serum with near-infrared transmission spectroscopy, Anal. Chim. Acta 371 (1998) 255–267.
[11] H.M. Heise, A. Bittner, Multivariate calibration for near-infrared spectroscopic assays of blood substrates in human plasma based on variable selection using PLS-regression vector choices, Fresenius J. Anal. Chem. 362 (1998) 141–147.
[12] S. Kasemsunram, Y.P. Du, K. Murayama, M. Huehne, Y. Ozaki, Near-infrared spectroscopic determination of human serum albumin, γ-glubulin, and glucose in a control serum solution with searching combination moving window partial least squares, Anal. Chim. Acta 512 (2004) 223–230.
[13] A. Bittner, R. Marbach, H.M. Heise, Multivariate calibration for protein, cholesterol and triglycerides in human plasma using short-wave near-infrared spectrometry, J. Mol. Struct. 349 (1995) 341–344.
[14] J.L. Pezzaniti, T. Jeng, L. McDowell, G.M. Oosta, Preliminary investigation of near-infrared spectroscopic measurements of urea, creatinine, glucose, protein, and ketone in urine, Clin. Biochem. 34 (2001) 239–246.
[15] S. Zhang, B.R. Soller, S. Kaur, K. Perras, T.J.V. Salm, Investigation of noninvasive in vivo blood hematocrit measurement using NIR reflectance spectroscopy and partial least-squares regression, Appl. Spectrosc. 54 (2000) 294–299.
[16] N. Kang, S. Kasemsumran, Y.W. Woo, H. Kim, Y. Ozaki, Optimization of informative spectral regions for the quantification of cholesterol, glucose and urea in control serum solutions using searching combination moving window partial least squares regression method with near infrared spectroscopy, Chemom. Intell. Lab. Syst. 82 (2006) 90–96.
[17] S. Kasemsumran, Y.P. Du, K. Maruo, Y. Ozaki, Improvement of partial least squares models for in vitro and in vivo glucose quantifications by using near-infrared spectroscopy and searching combination moving window partial least squares, Chemom. Intell. Lab. Syst. 82 (2006) 97–103.
[18] C.H. Petter, N. Heigl, R. Bakry, G.K. Bonn, A. Ritsch, C.W. Huck, Quantification of low-density and high-density lipoproteins in human serum by material enhanced infrared spectroscopy (MEIRS), Curr. Med. Chem. 16 (2009) 4601–4608.
[19] P.A.C. Filho, R.J. Poppi, Determination of triglycerides in human plasma using near-infrared spectroscopy and multivariate calibration methods, Anal. Chim. Acta 446 (2001) 39–47.
[20] L. Norgaard, A. Saudland, J. Wagner, J.P. Nielsen, L. Munck, S.B. Engelsen, Interval partial least-squares regression (iPLS): a comparative chemometric study with an example from near-infrared spectroscopy, Appl. Spectrosc. 54 (2000) 413–419.
[21] M. Ferrand, B. Huquet, S. Barbey, F. Barillet, F. Faucon, H. Larroque, O. Leray, J.M. Trommenschlager, Determination of fatty acid profile in cow's milk using mid-infrared spectrometry: Interest of applying a variable selection by genetic algorithms before a PLS regression, Chemom. Intell. Lab. Syst. 106 (2011) 183–189.
[22] M.C.U. Araujo, T.C.B. Saldanha, R.K.H. Galvao, T. Yoneyama, H.C. Chame, V. Visani, The successive projections algorithm for variable selection in spectroscopic multicomponent analysis, Chemom. Intell. Lab. Syst. 57 (2001) 65–73.
[23] P. Valderrama, J.W.B. Braga, R.J. Poppi, Variable selection, outlier detection, and figures of merit estimation in a partial least-squares regression multivariate calibration model. A case study for the determination of quality parameters in the alcohol industry by near-infrared spectroscopy, J. Agric. Food Chem. 55 (2007) 8331–8338.
[24] C. Arich, A. Gouby, C. Bengler, J.L. Ardilouze, A. Dubois, P. Joubert, S. Hansel, C. Janbon, S. Fabre, Comparison of the efficacy of cefotaxime alone and the combination cefazolin–tobramycin in the treatment of enterobacterial septicemia, Pathobiology 35 (1987) 613–615.
[25] M. Joshi, J. Bernstein, J. Solomkin, B.A. Wester, O. Kuye, Piperacillin/tazobactam plus tobramycin versus ceftazidime plus tobramycin for the treatment of patients with nosocomial lower respiratory tract infection. Piperacillin/tazobactam Nosocomial Pneumonia Study Group, J. Antimicrob. Chemother. 43 (1999) 389–397.
[26] R.W. Kennard, L.A. Stone, Computer aided design of experiments, Technometrics 111 (1969) 137–148.
[27] J.P. Fillastre, J. Hemet, P. Tulkens, J.P. Morin, G. Viotte, B. Olier, M. Godin, Comparative nephrotoxicity of four aminoglycosides: biochemical and ultrastructural modifications of lysosomes, Adv. Nephrol. Necker Hosp. 12 (1983) 253–275.
[28] A.M. Lerner, M.P. Reyes, L.A. Cone, D.C. Blair, W. Jansen, G.E. Wright, R. Lorber, Randomised, controlled trial of the comparative efficacy, auditory toxicity, and nephrotoxicity of tobramycin and netilmicin, Lancet 1 (1983) 1123–1126.