

Fast Direct Determination of Titanium Dioxide in Toothpastes by X-Ray Fluorescence and Multivariate Calibration

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Recentemente, o dióxido de titânio foi classificado como potencialmente carcinogênico pela International Agency for Research on Cancer (IARC). Dióxido de titânio é um pigmento geralmente utilizado como opacificante em cremes dentais, porém sua concentração não é indicada nos rótulos dos produtos. Neste estudo, 22 amostras de cremes dentais foram calcinadas a 800 °C e o teor de TiO₂ foi determinado por fluorescência de raios X por energia dispersiva (EDXRF) através do método de parâmetros fundamentais (FP). As mesmas amostras foram irradiadas *in natura* por 100 s e, através da correlação dos espectros e das concentrações anteriormente determinadas, um modelo multivariado de calibração foi construído. Oito variáveis latentes descreveram o modelo de regressão de mínimos quadrados parciais (PLS) com erros médios de 9,5%, indicando que além do pico referente ao titânio, as informações do espalhamento da radiação também são importantes para minimizar os erros ao usar uma calibração univariada. A rapidez das análises, com mínimo pré-tratamento das amostras, é a grande vantagem do método, que tem frequência analítica de 24 determinações por hora.

Recently, the International Agency for Research on Cancer (IARC) has classified titanium dioxide as potentially carcinogenic. Titanium dioxide is a pigment generally used as opacifying agent in toothpastes, but there is no indication of the percentage of this oxide in these products. In this work, 22 distinct toothpaste samples were calcinated at 800 °C and TiO₂ concentration was determined with energy dispersive X-Ray fluorescence (EDXRF) via fundamental parameter (FP) method. The same samples were irradiated *in natura* for 100 s and through the correlation of spectra and concentrations formerly determined, a multivariate calibration model was constructed. Eight latent variables described the partial least square regression (PLS) model with average errors of 9.5%, indicating that beyond the peak of titanium, the information of the X-Ray scattering irradiation is also important to minimize errors when using an univariate calibration. As a major advantage, the method allows analysis without pretreatment of the samples, with a throughput of 24 determinations *per hour*.

Keywords: toothpaste, titanium dioxide, X-ray fluorescence, partial least square regression, chemometrics

Introduction

Among the common habits used for tooth conservation, the frequent use of dentifrices, mainly in the form of pastes, can be considered the most practiced. Before being presented as pastes, the first dentifrices were commercialized as powders in 1850, in the United States of America.¹ Toothpaste popularization occurred when it was presented in flexible metallic tubes.² Nowadays, the chemical composition of toothpastes varies from one brand to another, and even among several presentations of

the same brand. They are composed by substances which act as abrasives, pigments, foam inducers, humectants, thickeners, stabilizers, solvents, sweeteners, therapeutic agents, enamel hardeners, etc. Other substances may be added, such as sugars, fragrances and flavorings.³

In general, TiO₂ is mostly used as pigment in paints, plastics, latexes, paper, textiles, food and drugs.⁴ It is also used as a pigment in toothpastes to confer a white color. The opacifying properties of this oxide render toothpastes a non translucent aspect.⁵ Titanium dioxide shows a high refractive index ($n = 2.7$), an aspect that is explored when this compound is used as physical protector against sun hazards to the skin since it reflects a great part of the sun

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radiation, including the ultraviolet wavelengths.⁶ This oxide in its anatase morphological form is also an effective catalyst in organic pollutant degradations,⁷ behaving as a light sensitive semiconductor.⁶⁻⁹ When used as pigment in cosmetic applications, it is named PW-6 or CI 77891.¹⁰ Around 70% of the world pigment production is related to TiO₂ manufacturing.¹¹

In 2006, the International Agency for Research on Cancer (IARC from World Health Organization, WHO) characterized TiO₂ as potentially carcinogenic to human beings. Other research shows that mice exposure to TiO₂ induces cancer in the respiratory tract of those animals. Recent data indicates that TiO₂-nanoparticles are cyto- and genotoxic against several lineages of cell cultures. They also present high carcinogenic potential in animal models.^{12,13}

Widespread use and its potential entry through dermal, ingestion and inhalation routes suggest that nanosized TiO₂ poses considerable exposure risk to humans, livestock and eco-relevant species,¹⁴ with growing concerns regarding the impact of TiO₂-nanoparticles spread throughout the environment¹⁵ due to the astonishing increasing of its production in recent years. It is a controversial subject that extensively attracts the attention of the scientific community. Consequently, the developments of fast and robust methods for TiO₂ analytical determinations are welcome.

In Brazil, the use of TiO₂ as an artificial dye (pigment) for ingestion is regulated by the Brazilian National Agency for Sanitary Vigilance (ANVISA).¹⁶ Nevertheless, no maximum allowed concentration is stated. The Food and Drugs Administration (FDA), an agency of the United States Department of Health and Human Services, determines 1% m/m as the maximum TiO₂ content when used as a food pigment.¹⁷

X-ray fluorescence (XRF) is an analytical technique related to the measurement of characteristic X-ray elemental energies and the corresponding intensities emitted by a sample after irradiation by high-energy particles or photons.¹⁸ Quantitative determinations can be performed since the observed emitted intensities are proportional to element concentrations.¹⁹ X-ray fluorescence may be regarded as a fast technique with low operational costs that simultaneously provides multielemental qualitative and quantitative results. It can be used to analyze inorganic and organic compositions.²⁰

These several positive features, besides being essentially non-destructive, render to XRF a large number of applications in chemistry, and also in studies involving medicine,²¹ geology,²² biology,²³ archeology,²⁴ food,²⁵ drugs²⁶ and environmental issues.²⁷ X-ray fluorescence is susceptible to interferences, the interelement effects being the most severe, caused by concomitant signal absorption and

intensification. The fundamental parameter (FP) method and chemometrics are examples of mathematical tools that are applied to overcome these drawbacks.²⁸

Fundamental parameter method correlates the intensity of a given emission line to the concentration of the fluorescent element without using standards, *a priori*. In comparing emissions to absorption methods, that are ruled by the Beer-Lambert law ($A = \epsilon LC$), FP in XRF calculates the corresponding term associated with ϵ , avoiding the need for standards. For XRF, FP is really useful in correcting matrix effects since each line intensity is not directly proportional to concentration, but is affected by other elements in the sample.²⁹ Fundamental parameter is nowadays regarded as a state-of-art method for overcoming matrix effects and is based on iterative mathematical calculations with no concentration standards, two strong points that make use of FP widespread. Nevertheless, FP presents a drawback related to the uncertainty in some values employed, such as mass absorption coefficients and fluorescence yields, aspects that can seriously impair the calculations, mainly if the sample under analysis is too complex.³⁰

Chemometrics or multivariate calibration is considered an important tool for analytical chemistry since it works only with non-correlated spectral variables having relevant information. The combination of XRF and multivariate calibration furnishes excellent solutions, as it can minimize or even eliminate analytical steps, mainly sample preparation.³¹⁻³³ Partial least square regression (PLS) is the most applied chemometric tool in quantitative analysis. It initially consists in calculating a relation between a data matrix (as a spectra set) and reference properties (as concentrations), using only a low number of spectral variables that carry relevant chemical information. To ease interpretation, original data pre-processing is often applied, with mean centering being the most common one for spectroscopic studies.³⁴

In recent works involving TiO₂ determinations, Melquiades *et al.*⁶ described an EDXRF method applied to sunscreens, using an analytical curve with previously prepared standards. Colquitt³⁵ quantified several metallic oxides in toothpastes, using sample ashing and inductively coupled plasma optical emission spectrometry (ICP OES).

In this work, a new cooperation between XRF and PLS modeling is developed in order to quantify TiO₂ in dentifrices without sample preparation.

Experimental

Experimental procedure

Twenty-two commercial toothpaste samples of different brands having diverse presentations were acquired in the

local stores of Campinas City, São Paulo State, Brazil. All sample labels indicate the presence of TiO_2 , but not its actual content.

The experimental procedure for acquiring and validating the PLS model involved seven steps, as described below.

Sample calcination

A mass of 10 g (± 0.00001 g) of each toothpaste, in triplicate, weighted in porcelain cups, was dried at 130 °C for 2 h, and then calcinated at 800 °C for 4 h. After cooling in a desiccator, the cups were weighted on an analytical balance (Ohaus, model Analytical Plus) and the ashes were ground.

Ash sample irradiation

Ash samples were transferred to XRF sample cells (Chemplex 1330), prepared with their bottoms sustained by 2.5 μm width Mylar® films and irradiated for 100 s in triplicate in a Shimadzu EDX-700 spectrometer with a rhodium target X-ray tube and a Si(Li) detector. The applied voltage for the X-ray tube was 50 kV and the detector dead time 25%. The spectra were sequentially acquired from 0 to 40 keV with energy steps of 0.02 keV. The maximum current accepted in the tube can reach 100 μA , but its actual value is regulated by the dead time of the detector, to avoid its saturation.

Fundamental parameter analysis of ash samples to determine TiO_2 contents

Fundamental parameter delivers quantitative sample compositions from the intensities of the analyte lines and known values of three fundamental parameters: (i) primary spectral distribution (source), (ii) mass and photoelectric absorption coefficients and (iii) fluorescence yield. The absorption coefficient (μ) is a constant related to the loss of fluorescence when the radiation crosses the sample divided by the sample width. However, the mass absorption coefficient (μ_m) is a function of μ divided by the material density, being a more useful value.²⁸⁻³⁰ Equation 1 shows how FP is applied for a very thin sample excitation by monochromatic radiation, calculated by software DXP-700E (version 1.0).

$$I_A = I_0 \omega_A g_A \frac{r_a - 1}{r_a} \frac{d\Omega}{4\pi} \frac{C_A \mu_A(\lambda_{\text{prim}}) \text{cossec } \varphi}{\mu_M(\lambda_{\text{prim}}) \text{cossec } \varphi + \mu_M(\lambda_A) \text{cossec } \psi} \quad (1)$$

where: I_A is the line intensity of analyte A; I_0 is the primary beam intensity at the wavelength λ_{prim} ; λ_{prim} is the effective wavelength of the primary X-ray beam; ω_A is

the fluorescence yield for element A; g_A is the fractional value of line in the analyte series; r_A is the absorption edge for element A; $d\Omega/4\pi$ is the fractional value of the fluorescent X-ray beam directed to the detector; C_A is the concentration of element A; $\mu_A(\lambda_{\text{prim}})$ is the mass absorption coefficient of A at λ_{prim} ; $\mu_M(\lambda_{\text{prim}})$ is the mass absorption coefficient of matrix at λ_{prim} ; $\mu_M(\lambda_A)$ is the mass absorption coefficient of matrix at λ_A ; φ is the incidence angle of the primary X-ray beam; ψ is the exit angle of the fluorescent beam.

Titanium dioxide contents by FP in ash samples (C_{ashes}) were then calculated. The concentration in toothpastes (C_{paste}) is available considering Equation 2, where m_{paste} is the toothpaste mass before calcination and m_{ashes} is the ash mass. C_{paste} values were then considered as reference values for each sample used in the PLS model.

$$C_{\text{paste}} = \frac{m_{\text{ashes}}}{m_{\text{paste}}} C_{\text{ashes}} \quad (2)$$

In this calculation of reference values, TiO_2 concentrations are taken as proportional to the determined Ti concentrations since it is presumed that all titanium atoms are in the form of TiO_2 in the toothpastes.

Accuracy evaluation of FP method

To evaluate the accuracy of the FP method for C_{ashes} , recovery tests were applied. The reagents were previously dried at 130 °C for 2 h and left to cool in a desiccator. Masses of pure TiO_2 (Riedel-deHaën) were weighted and completed with silica to 1 g (SiO_2 , Merck). The concentrations of the prepared standards of TiO_2 in silica were 0.25, 0.50, 1.00, 2.00, 3.00 and 3.50 g 100 g⁻¹. They were ground, deposited in the XRF cells and irradiated, as described in Ash sample irradiation section.

Direct toothpaste irradiation

Homogeneous toothpaste samples (with no prior pretreatment) were also irradiated as described in Ash sample irradiation section. Heterogeneous samples previously homogenized in a beaker with the aid of a glass rod, and then transferred to the XRF cells (Figure 1).

Partial least square regression modeling of toothpaste samples for TiO_2 determination

X-ray fluorescence spectra of 16 samples were used to build the multivariate calibration PLS model, being the mean values obtained in Direct toothpaste irradiation section taken as TiO_2 reference concentrations. Data



Figure 1. Same toothpaste sample, before and after homogenization.

treatments were performed with the aid of the software Pirouette® 3.11 (Infometrix Co.).

Partial least square regression validation and figures of merit

From the set of 22 samples in triplicate (66 spectra), 16 (48 spectra) were used for the PLS modeling (PLS modeling of toothpaste samples for TiO₂ determination section) and the remaining 6 (18 spectra) for external validation. To statistically evaluate the PLS model, some figures of merit were calculated, with the aid of the software Matlab® 6.5 (MathWorks).

Results and Discussion

After calcination, the samples presented colors ranging from white to light gray. Ash mass variations ranged from 68 to 88% since the samples were of different brands and presentation types.

Table 1 shows the mean C_{ashes} obtained by the FP method and C_{paste} , calculated in accordance to Equation 2. C_{paste} values vary from 0.04 to 0.87 g 100 g⁻¹.

The accuracy in FP determinations of C_{ashes} values was undertaken by recovery tests. Silica was used as matrix in the standards since this oxide is the most abundant in toothpaste ashes. After irradiation, FP was applied and Table 2 presents the recovery results.

The recovery values of TiO₂ content in silica determined by FP was about 100%, thus eliminating the need of an analytical curve to acquire them. The application of FP is reliable and very common when applied to simple matrices such as ashes³⁶⁻³⁸ since it minimizes the interferences due to interelement absorption/intensification. Nevertheless, the time spent in sample calcination (8 h) is not feasible in routine analysis, justifying a search for a direct determination of TiO₂ content in toothpastes. In addition, direct FP in complex samples is not viable, providing uncertainty in the theoretical and geometrical parameter values. On the other hand, chemometric methods are based on models built with calibration standards, when uncertainty related to FP theoretical parameters of the

Table 1. C_{ashes} and C_{paste} values (mean \pm standard deviation, n = 3)

Sample	$C_{\text{ashes}} / (\text{g } 100 \text{ g}^{-1})$	$C_{\text{paste}} / (\text{g } 100 \text{ g}^{-1})$
TP1	3.6 \pm 0.1	0.87 \pm 0.03
TP2	3.0 \pm 0.1	0.71 \pm 0.03
TP3	2.57 \pm 0.07	0.66 \pm 0.02
TP4	1.96 \pm 0.01	0.503 \pm 0.003
TP5	2.36 \pm 0.01	0.445 \pm 0.002
TP6	3.3 \pm 0.1	0.41 \pm 0.01
TP7	1.89 \pm 0.03	0.385 \pm 0.005
TP8	1.073 \pm 0.007	0.345 \pm 0.002
TP9	1.45 \pm 0.01	0.353 \pm 0.002
TP10	1.179 \pm 0.008	0.336 \pm 0.002
TP11	1.13 \pm 0.06	0.24 \pm 0.01
TP12	0.95 \pm 0.02	0.222 \pm 0.004
TP13	0.76 \pm 0.02	0.171 \pm 0.005
TP14	0.69 \pm 0.04	0.143 \pm 0.008
TP15	0.55 \pm 0.01	0.129 \pm 0.003
TP16	0.776 \pm 0.009	0.102 \pm 0.001
TP17	0.39 \pm 0.01	0.081 \pm 0.003
TP18	0.211 \pm 0.003	0.049 \pm 0.007
TP19	0.200 \pm 0.009	0.047 \pm 0.002
TP20	0.234 \pm 0.009	0.054 \pm 0.002
TP21	0.271 \pm 0.009	0.052 \pm 0.002
TP22	0.249 \pm 0.002	0.045 \pm 0.004

Table 2. Results of recovery tests for FP applied to ash spectra (mean \pm standard deviation, n = 3)

TiO ₂ standard / (g 100 g ⁻¹)	TiO ₂ by FP / (g 100 g ⁻¹)	Recovery / %
0.25	0.27 \pm 0.03	108
0.50	0.55 \pm 0.09	110
1.00	1.05 \pm 0.05	105
2.00	2.00 \pm 0.07	100
3.00	2.95 \pm 0.04	98
3.50	3.47 \pm 0.09	99

sample matrix and geometrical instrumental characteristics are avoided. Besides, with chemometrics, one can quantify species even in the case of severe interference occurring simultaneously, common drawbacks of XRF.

To construct the calibration and validation models for TiO₂ in toothpastes, the spectra of these samples (Figure 2) were submitted to PLS, having as X-matrix the set of spectra (16 lines and 2048 columns) and as Y-matrix, the concentration values obtained by FP after calcination (Table 1).

In the constructed model, the only preprocessing employed was data mean centering, a process that subtracts

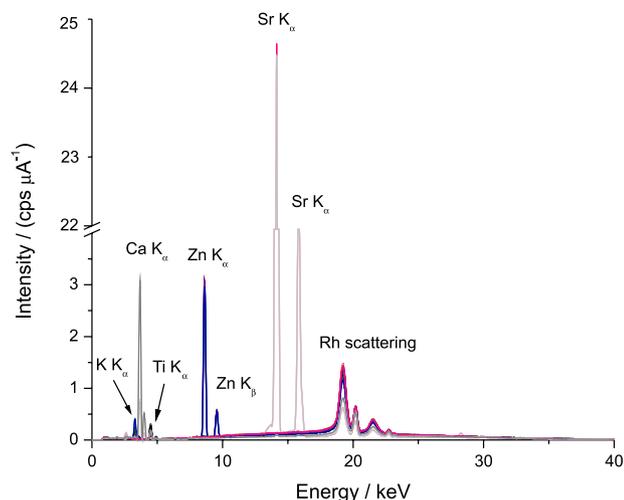


Figure 2. Direct X-ray spectra of 16 toothpaste samples of the PLS calibration set.

the element in each column from the mean value of all elements of the same column, resulting in a matrix where all columns present zero as mean.³⁹ To minimize signal noise, the spectra were smoothed by movable media, with a window of 15 points. The calibration model was improved by first choosing the minimum number of latent variables (LV) and then the minimum error of cross validation. Finally, outliers were identified and taken out of the model.

The minimum number of LV was chosen on the basis of predict residual error sum of squares (PRESS) values, corresponding to the sum of squared prediction errors, calculated for each LV.⁴⁰ As shown in Figure 3, PRESS values are constant from the eighth LV. So, the number of LV to build the model was 8, explaining 99.99% of the accumulated variance.

Generally speaking, loading graphs show the magnitude each linear combination of non-correlated variables (called

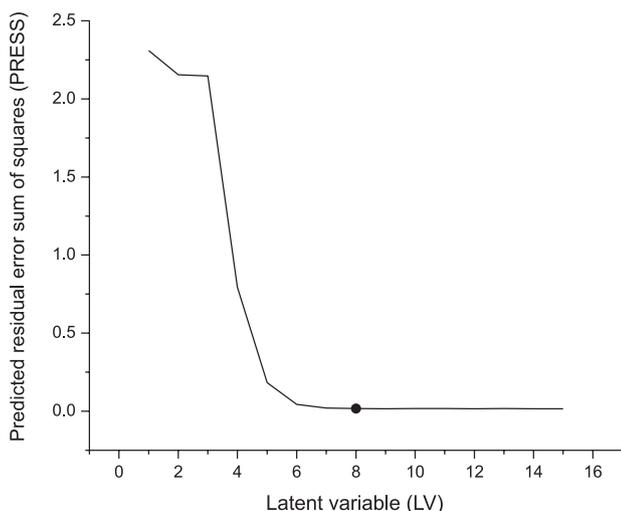


Figure 3. Predicted residual error sum of squares (PRESS) as function of the number of latent variables (LV).

latent variables, LV) contributes to the total explained variance of the PLS model, with the first LV carrying most of the explained variance. For this work, Figure 4 shows the loadings graph for the eight LV and very interesting interpretations can be taken from these results. The first and second LV (92.5 + 5.4% of total explained variance) are surprisingly not affected by the Ti K_α peak, but by heavier elements, Sr and Zn. They emit photons with higher energy than Ti K_α absorption, proportionally increasing the Ti K_α intensity, a phenomenon well known as signal intensification. Only the third LV begins to present variance related to Ti K_α peak.

For the remaining five LV, the opposite physical effect can be indicated. Elements lighter than Ti, such as Ca and K, absorb energy from Ti K_α peak, reducing its intensity, a phenomenon called interelement absorption. X-ray Rh K scattering (from the X-ray source), between 19 and 22 keV, also contributes, from the second to the eighth LV, an aspect related to variations in densities and organic compositions of toothpastes. Therefore, for TiO₂-PLS modeling using directly spectra of toothpastes, a great amount of information is obtained, not only variations in the Ti K_α peak, but mainly those related to interelement interferences (absorption/intensification) and variations in the organic composition of toothpastes.

To illustrate these findings, a conventional univariate analytical curve using only Ti K_α intensities for 16 samples (Figure 5) is compared to the PLS model built here (Figure 6).

Figure 5 is not useful, given its poor regression coefficient (0.843) and low prediction capacity, evaluated by root mean square error of prediction (RMSEP), equal to 0.17 g 100 g⁻¹. The PLS model (Figure 6) is robust and presents adequate calibration and cross validation regression coefficients, equal to 0.998 and 0.996, respectively.

Table 3 shows the mean predicted values and the errors determined for each value in applying the full cross validation process, with the prediction errors being lower than 13 g 100 g⁻¹. One replicate spectrum of each of the following samples was taken out of the PLS model, for being outlier, with elevated Student residues: TP5, TP13, TP14 and TP20.

To statistically check the PLS model, some figures of merit were determined as proposed by Valderrama *et al.*,⁴¹ and these values are presented in Table 4.

The limits of detection (LOD) and quantification (LOQ) are below the lowest measured value for TiO₂ concentration in all samples. The *t*-test, in turn, allowed concluding that the systematic errors in the model are not significant and can be disregarded since the calculated

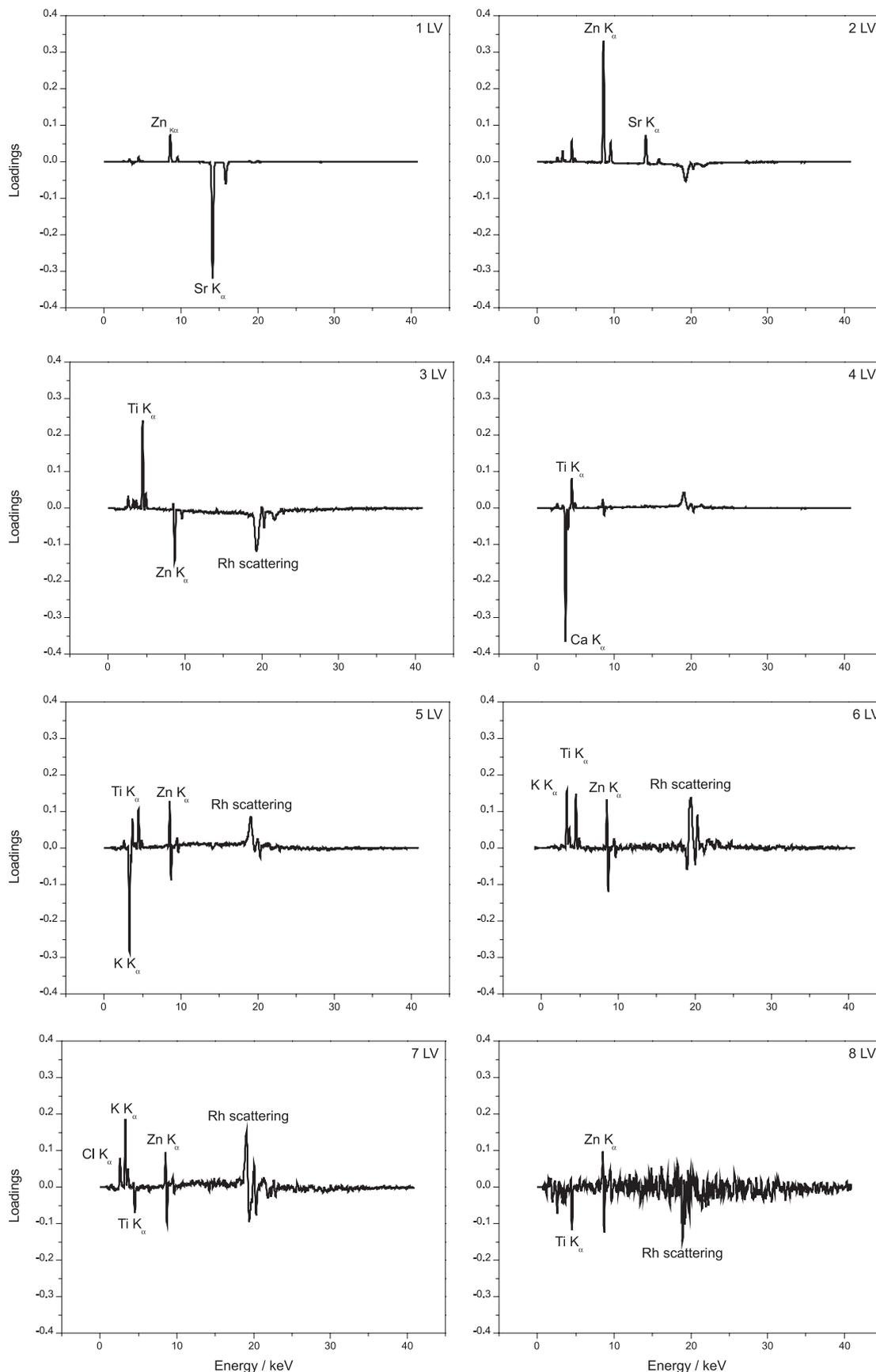


Figure 4. Loading graphs for the eight latent variables (LV) of the TiO₂-PLS model.

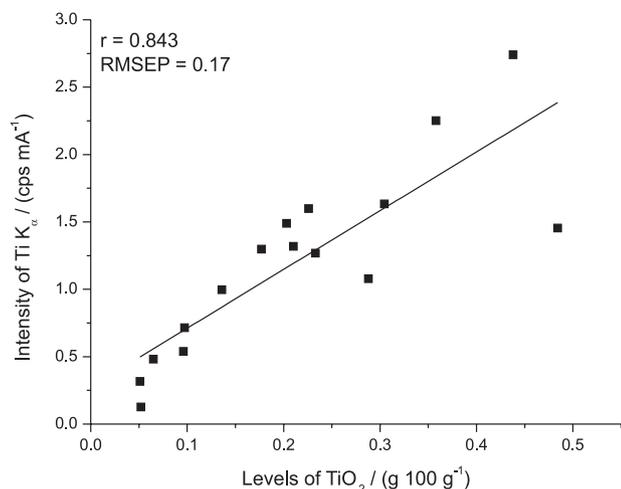


Figure 5. Intensity of Ti K_{α} peak vs. Ti concentration, after direct irradiation of 16 toothpaste samples (univariate calibration).

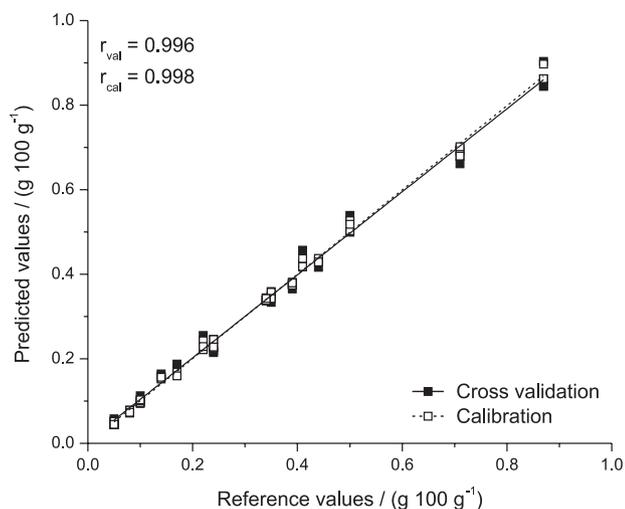


Figure 6. Correlation curves for predicted vs. reference TiO_2 values for calibration and full cross validation (multivariate method).

t_{bias} (0.012) is lower than the theoretical t_{bias} (2.31), with a confidence level of 95%. The model ability to calibrate and to predict is satisfactory since the values of root mean square error of calibration (RMSEC) and of root mean square error of prediction (RMSEP) were low, 0.0120 and 0.0283%, respectively.

The triplicates of samples TP3, TP8, TP15, TP18, TP19 and TP22 were selected for external validation as they are representative of the six distinct brands found on the Brazilian market. In Table 5, the mean prediction errors and the errors obtained in external validation are presented.

The maximum value error in the external validation was 16% for sample TP3, with a mean error of 9.5%, being therefore evident that the PLS model is recommended for the direct quantitative determination of TiO_2 in commercial toothpastes by joining XRF and chemometrics.

Table 3. TiO_2 reference and predicted values with mean prediction errors in internal validation using the PLS model

Sample	Reference value / (g 100 g ⁻¹)	Predicted value / (g 100 g ⁻¹)	Error / %
TP1	0.870	0.865	1
TP2	0.710	0.679	4
TP4	0.503	0.522	4
TP5	0.445	0.426	4
TP6	0.409	0.435	6
TP7	0.385	0.373	3
TP9	0.353	0.346	2
TP10	0.336	0.340	1
TP11	0.242	0.227	6
TP12	0.222	0.238	7
TP13	0.171	0.182	6
TP14	0.143	0.162	13
TP16	0.102	0.101	1
TP17	0.0812	0.0748	8
TP20	0.0539	0.0509	6
TP21	0.0522	0.0470	10

Table 4. Figures of merit of PLS model in quantitative determinations of TiO_2 in toothpastes

Figures of merit	Value
RMSEC	0.012 ^a
RMSEP	0.028 ^a
LOD	0.013 ^a
LOQ	0.039 ^a
t_{bias}	0.012 ^b

^ag 100 g⁻¹; ^b $t_{table} = 2.31$ (95% confidence).

Table 5. TiO_2 reference and predicted values with mean prediction errors in external validation using the PLS model

Sample	Reference value / (g 100 g ⁻¹)	Predicted value / (g 100 g ⁻¹)	Error / %
TP3	0.655	0.548	16
TP8	0.345	0.329	5
TP15	0.129	0.135	4
TP18	0.0496	0.0439	12
TP19	0.0474	0.0528	11
TP22	0.0408	0.0370	9

Conclusions

The proposed method involving toothpaste XRF spectra and chemometrics is dedicated to quantify TiO_2 , using as reference values those acquired by applying FP

to the spectra of ashes of the same samples. The use of eight latent variables is justified since the great diversity among sample matrices to attend specific aims, such as dental plaque prevention or teeth whitening. This vast diversification among sample compositions leads to a corresponding diversity in sample spectra, affecting titanium fluorescence signals in several non-correlated aspects. It is worthwhile mentioning that the method works well only for samples containing titanium since samples with no TiO₂ were not considered in the model construction.

Sample TP1 presented the highest TiO₂ content (0.87 g 100 g⁻¹), but the pertinent legislation agencies do not establish a safe upper limit for the use of this pigment in products for mouth hygiene. Nevertheless, 1 g 100 g⁻¹ is the maximum value recommended by FDA for food.¹⁷

Finally, the method introduced here is able to predict values for external samples with mean errors of 9.5%. Whereas the calcination/ash-irradiation/FP-determination takes 8 h to be concluded, the PLS model delivers results in 5 min (around 100-fold decreasing in analytical time). From this, it can be asserted that the alliance of XRF and chemometrics is very effective for the direct quantitative determination of TiO₂ in commercial toothpastes, in a fast, non-destructive procedure, generating no residue and with a minimal sample preparation.

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