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Potentiometric determination of nitrate in products used for the treatment of dentinal hypersensitivity

Ricardo Pérez-Olmos [a,*], Pilar Bezares [a], Javier Pérez [b]

[a] *Departamento de Química Analítica, EUITI, Universidad del País Vasco, Plaza de la Casilla no. 3, E-48012 Bilbao, Spain*
[b] *Departamento de Ingeniería Química y del Medio Ambiente, EUITI, Universidad del País Vasco, Plaza de la Casilla no. 3, E-48012 Bilbao, Spain*

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Abstract

In this paper, a rapid, simple and inexpensive potentiometric method for the determination of the potassium nitrate contained in different dentinal desensitizer products is described. The nitrate-selective electrode, using a tetraoctylammonium salt as sensor, was constructed without an internal filling solution. The ionic strength of the sample solutions was fixed by means of a 0.33 mol l^{-1} K_2SO_4 solution and direct potentiometry was used as the analytical technique of measurement. Precision and accuracy of the method developed were adequate since the mean of the relative standard deviations was 1.2% and the average percentage of spike recoveries was 100.9%. Eleven samples of toothpastes, gels and mouthwashes were analyzed by the proposed potentiometric method and the quality of the results was compared by means of linear regression analysis with those obtained by high-performance liquid chromatography (HPLC), adopted as the reference technique. Satisfactory agreement was observed. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Dentinal desensitizer analysis; Nitrate determination; Ion-selective electrodes

1. Introduction

Many people complain of having teeth that are sensitive to hot or cold liquids or to tactile stimuli. Dentinal hypersensitivity is characterized by more severe pain responses to stimulus levels that may not cause discomfort in the normal tooth. The roots of teeth are usually covered by gum tissues, but with time, attrition, abrasion, and erosion the thin outer layer of cementum is removed, exposing the underlying porous dentine. Root surface exposure allowing contact with the stimuli may also be caused by gingival recession or may be a consequence of some types of periodontal treatment. These stimuli may elicit pain because dentine tubules contain fluid and hot or cold stimuli can cause this to expand or shrink, stimulating the underlying pulpal nerves and resulting in pain [1].

It is clear that a potential desentitizing agent could act by reducing fluid flow through the dentine by clogging the tubules and decreasing the activity of the dentinal sensory nerves. Recently, several studies with canine teeth of anesthetized cats and dogs [2] and human volunteers [3–5] have been carried out and from the results obtained it is possible to affirm that potassium nitrate is an effective desensitizing agent. Toothpastes, gels and mouthwashes containing potassium nitrate have recently been marketed in several countries, but unfortunately no analytical method for its control has been published. For that reason it is important to develop analytical procedures for the determination of the potassium nitrate contained in these products.

Potentiometry with ion-selective electrodes has the advantage of allowing analysis of samples with a heterogeneous composition. In addition to their selectivity, the electrodes are characterized by a high linearity range. So it was decided to develop an economic, rapid and simple method based on the use of a nitrate-selective electrode for the determination of nitrate in toothpastes, gels and mouthwashes. Potentiometric determinations were carried out by using a nitrate-selective electrode without internal reference solution, con-

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[*] Corresponding author.

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structed according to the process described elsewhere [6,7], in which the sensor in a PVC matrix was applied directly on a conductive support.

A 0.33 mol l^{-1} K_2SO_4 solution for adjusting the ionic strength has been used. Certain tensoactive agents contained in some products interfere in the membrane response, so different organic extractions and analytical techniques of measurement have been carried out and the precision and accuracy of the proposed method have been established.

Since this is the first time that nitrate has been determined in this type of products, it was thought that it would be important to compare the results obtained by potentiometry with those supplied by other instrumental techniques. Some UV spectrophotometric methods are handicapped because they are time-consuming and lacking in selectivity and reproducibility. For that reason, high-performance liquid chromatography (HPLC) has been adopted as the reference technique.

In this work, the chromatographic determinations have been carried out using an aminocolumn, with photometric detection and a 5.0×10^{-3} mol l^{-1} (pH 3.5) KH_2PO_4 solution as eluent. Eleven samples of different desensitizer agents have been simultaneously analyzed by the proposed and reference methods and the results obtained compared by means of a linear regression analysis.

2. Experimental

2.1. Apparatus and electrodes

The preparation of the body of the electrode and the application of the conductive support, formed by a mixture of Araldite and graphite powder, were carried out as described elsewhere [6]. The membrane of the nitrate-selective electrode was prepared as previously reported [7], by using tetraoctylammonium bromide (sensor), dibutylphthalate (solvent mediator) and PVC previously dissolved in tetrahydrofuran (inert matrix). The electrode was constructed directly applying the membrane onto the solid conductive support, according to the process described elsewhere [7]. The potentiometric measurements were performed with an Orion 710 digital pH/mV meter (sensitivity ± 0.1 mV), using an Orion 90-02-00 double junction AgCl/Ag reference electrode. For pH determinations an Orion 91-02 combined glass electrode was used.

The liquid chromatography equipment used was a Waters 490E ultraviolet–visible programmable multiwavelength detector, two Waters 510 pumps, and a Phase Separations Inc. spherisorb S_5-NH_2 (250×4.6 mm), 5 μm particle size, column. For the equipment control and data acquisition a NEC, Power Mate 3B6125 microcomputer was used. The data were treated by means of a Maxima 820 program designed by Waters.

2.2. Reagents

The nitrate standard solution was a 0.01 mol l^{-1} $NaNO_3$ solution prepared after careful weighing of the solid reagent, previously oven dried for 2 h at 120°C, and stored in a polyethylene bottle. The ionic strength adjuster (ISA) solution was a 0.33 mol l^{-1} ($I = 1.0$ mol l^{-1}) K_2SO_4 solution. The inner chamber filling solution for the reference electrode was prepared from 1.70 mol l^{-1} KNO_3, 0.64 mol l^{-1} KCl and 0.06 mol l^{-1} NaCl; this solution was saturated with AgCl. The outer chamber filling solution for the reference electrode was a 0.033 mol l^{-1} ($I = 0.1$ mol l^{-1}) K_2SO_4 solution. The mobile phase used for the chromatographic determinations was a 5.0×10^{-3} mol l^{-1} (pH 3.5) KH_2PO_4 solution filtered through a Millipore 0.45 μm filter. Distilled and deionized water with a conductivity less than 0.1 μS cm^{-1} and analytical grade reagents were used throughout.

3. Procedures

3.1. Sample treatment

A toothpaste or gel sample (1 g) was weighed exactly and placed into a 100 ml beaker, 5 ml of a 4 mol l^{-1} H_2SO_4 solution were added and the solution was stirred and heated (70°C) for 30 min. Afterwards, 20 ml of deionized water were added, the beaker was covered with a watch-glass and the solution was gently boiled for 1 h. After cooling, the solution was transferred to a 500.0 ml volumetric flask and the volume was completed with deionized water. This solution was filtered and an aliquot of 100.0 ml was transferred to a separating funnel where 50 ml of ethyl ether and 50 ml of ethylacetate were added. After shaking, the aqueous phase was collected and the pH was adjusted to 3.5–4.0 by means of a 5 mol l^{-1} NaOH solution.

In the case of the mouthwashes it was not necessary to carry out the organic extraction, but a previous debubbling and filtration of a few volumes of the samples, to avoid foam formation, was recommended. An exact volume (1.0 ml) of the filtered solution was pipetted into a 500.0 ml volumetric flask and made up to volume with deionized water.

3.2. Proposed potentiometric procedure

The treated sample solution (50.0 ml) was pipetted into a 100 ml beaker and 5.0 ml of the ISA solution were added. The nitrate-selective and reference electrodes were immersed in this solution and after waiting for a stable reading of potential, the nitrate concentration was determined by direct potentiometry.

3.3. *Chromatographic reference procedure*

An aliquot of the extracted sample solution was filtered through a Whatman Anotop 25 Plus filter and 10 μl of this solution were injected into the HPLC equipment. The separation and detection were carried out according to the following chromatographic conditions, which were similar to those reported elsewhere [8]: elution mode, isocratic; flow rate, 3.0 ml min^{-1}; detection wavelength, 215 nm; temperature, ambient; absorbance units full scale, 0.2; retention time expected, 7.3 ± 0.2 min; analysis time, 10.0 min.

4. Results and discussion

It is well known that toothpastes and gels are usually dissolved in hydrochloric acid for their analysis, but on this occasion this acid cannot be used because of the interference of the chloride ion on the response of the nitrate-selective electrode. For this reason, in this work the use of a 4.0 mol l^{-1} H_2SO_4 solution has been adopted. Unfortunately, when different samples of these products were dissolved in this solution, diluted with deionized water and stirred, foam was produced and consequently a shift in the potential values was observed. To avoid this problem, several dilution ratios (1:10, 1:50, 1:100 and 1:500) were studied and it has been proved that the best results were obtained with the highest dilution ratio.

A 0.33 mol l^{-1} K_2SO_4 solution was adopted as ISA solution and the influence of ionic strength on potential values was studied. The minimum volume of ISA solution needed for samples of toothpastes, gels and mouthwashes was obtained from plots of potential values versus volume of the ISA solution added to 50.0 ml of samples, which had been treated as described in Section 2. From the plots obtained it is possible to affirm that the addition of 5.0 ml of ISA solution is adequate. In these conditions the lower limit of linear response was found to be 6.5×10^{-5} mol l^{-1} (with a practical limit of detection of 2.0×10^{-5} mol l^{-1}) and the upper limit was 1.0×10^{-1} mol l^{-1}.

It has been observed that there is no influence from chlorides on the membrane response at the concentration usually found in these products. The potential interference from carbonate and hydrogen carbonate is easily avoided if the sample solutions are adjusted to pH 3.5–4.0 before the potential values are measured. Moreover, these compounds would be initially removed when toothpastes and gel samples are dissolved in sulfuric acid. On the contrary, the presence of tensoactive agents in some samples of toothpastes and gels causes a drift in the potential values. For that reason liquid extractions studies, using different organic solvents, have been performed. Initially, extractions of the treated sample solutions with hexane, heptane, xylene, carbon tetrachloride, ethyl ether or ethyl acetate in different sample:solvent ratios were carried out, but unsatisfactory results were obtained. Afterwards, assays using different mixtures and ratios of these solvents were performed and the best result was achieved by using a mixture of 50% ethyl ether and 50% ethyl acetate in a sample:solvent ratio of 1:1.

A study has been carried out to select the most suitable potentiometric technique of measurement. The nitrate contained in toothpastes, gels and mouthwashes was determined by using direct potentiometry, the standard additions and the sample addition methods, and the precision and accuracies of all techniques were calculated. The precisions were measured by application of the three potentiometric techniques to 11 samples of the same product. The mean concentrations, the standard deviations, the relative standard deviations and the percentages of relative errors were established. Nitrate standard additions were used to evaluate the accuracies of the techniques. Four identical samples of the same product were prepared, and their nitrate contents were determined by all the potentiometric techniques. One sample received no added nitrate, and additions of 1, 2, 3 ml of a 0.25 mol l^{-1} potassium nitrate standard solution were made to the other three samples before being diluted to 500 ml, and the percentages of spike recoveries were determined. From the data obtained it was possible to ascertain that there was no significant difference between the values obtained by all the techniques. For this reason, direct potentiometry, the easiest and fastest method, has been adopted as the technique of measurement.

As the authors could not find any analytical method in the literature for the determination of nitrate in products used against dentinal hypersensitivity, the same treated sample solutions, previously mentioned, were simultaneously analyzed by adopting HPLC as the reference technique. Following the chromatographic reference procedure, a linear calibration in the concentration range of 20–140 mg l^{-1} could be established.

Finally, six samples of toothpastes, two samples of gels and three samples of mouthwashes, purchased in several European countries, were simultaneously analyzed by both methods and the results obtained are shown in Table 1. From these data it is possible to affirm that the precision and accuracy of the potentiometric procedure developed were good since the mean relative standard deviation was 1.2% and the average percentage of spike recovery resulted to be 100.9%. When the reference method was applied, these resulting values were 0.9 and 102.3%, respectively. The limits of detection of the proposed potentiometric method, es-

Table 1
Results obtained in the determination of nitrate in dentinal desensitizer agents by simultaneous application of the proposed potentiometric and chromatographic reference methods [a]

Sample	KNO_3 labelled concentration	Nitrate determination							
		ISE			HPLC				
		X [d]	RSD [e]	R [f]	X [d]	RSD [e]	R [f]	RE [g]	F-value [h]
T1	50 [b]	51.3 ± 0.3	0.6	101.1	52.2 ± 0.2	0.4	103.5	−1.7	2.25
T2	50 [b]	48.7 ± 0.5	1.0	98.5	48.2 ± 0.4	0.8	99.8	+1.0	1.56
T3	50 [b]	49.8 ± 0.4	0.8	101.9	51.5 ± 0.3	0.6	102.7	−3.3	1.78
T4	50 [b]	50.1 ± 0.2	0.4	103.8	49.8 ± 0.3	0.6	99.5	+0.6	2.25
T5	50 [b]	48.0 ± 0.4	0.8	102.1	50.2 ± 0.2	0.4	102.8	−4.4	4.00
T6	50 [b]	49.9 ± 0.2	0.4	101.5	50.1 ± 0.2	0.4	102.6	−0.4	1.00
G7	50 [b]	48.8 ± 0.3	0.6	99.6	49.1 ± 0.4	0.8	101.9	−0.6	1.78
G8	50 [b]	50.1 ± 0.6	1.2	98.8	51.0 ± 0.5	1.0	102.5	−1.8	1.44
M9	50 [c]	49.7 ± 0.5	1.0	100.7	51.0 ± 0.3	0.6	103.9	−2.5	2.78
M10	10 [c]	10.2 ± 0.3	2.9	101.4	10.3 ± 0.2	1.9	102.9	−1.0	2.25
M11	10 [c]	10.3 ± 0.4	3.9	100.7	10.4 ± 0.3	2.9	103.4	−1.0	1.78

[a] T1–T6, toothpastes; G7–G8, gels; M9–M11, mouthwashes.

[b] g kg^{-1}.

[c] g l^{-1}.

[d] Mean nitrate concentration and standard deviation (g kg^{-1} for toothpastes and gels, g l^{-1} for mouthwashes.

[e] Relative standard deviation (%).

[f] Mean spike recovery (%).

[g] Relative error of the potentiometric method versus the reference method (%).

[h] The critical F-value considering a 95% of confidence level and 10 degrees of freedom, for a two-tailed F test, is 3.72.

tablished according to the definition of the Analytical Methods Committee [9], when applied to the treated samples of toothpastes and gels or mouthwashes, were 2.1 and 1.6 mg l^{-1} respectively.

To test whether the proposed and reference methods differ in their precision, a significance F-test (two-tailed test) was carried out. The calculated F-values for all the samples analyzed were less than the critical F-value, with the exception of one toothpaste sample; so there is no significant difference between the two standard deviations at the 95% confidence level.

From the results obtained it is also possible to ascertain that adequate agreement between both methods was found, with a slight tendency to get lower values with the proposed potentiometric method than with the chromatographic reference method. The highest difference between results obtained for the samples was −4.4% and the lowest was +0.6% with an average value of −1.4%. The results obtained have also proved to be in agreement with the concentration labelled in these dentinal desensitizing products.

A relation of the type $C_{\mathrm{p}} = C^\circ + sC_{\mathrm{r}}$ was established between the results obtained with the proposed potentiometric method (C_{p}) and those obtained with the chromatographic reference method (C_{r}) when a linear regression analysis was applied. Values of $+0.01$ for C°, 0.98 for s and 0.997 for the correlation coefficient were obtained. The confidence limits of the slope (0.02), intercept of the regression line (0.07) and the t-value for the correlation coefficient (4.74) at a 95% confidence level and $n-2$ degrees of freedom were also calculated. From these data it is possible to affirm on the one hand, that the calculated slope and intercept do not significantly differ from the ideal values of 1 and 0, respectively and thus that there is no evidence for systematic differences between both analytical methods. On the other hand, the calculated t-value was greater than the tabulated t-value (2.26), so it is possible to state that significant correlation exists between the proposed and reference methods [10].

5. Conclusions

The results obtained in this work have proved that, from the perspective of quality control, the use of a nitrate-selective electrode may be considered as an advantageous analytical method since it is easier to operate and requires less expensive equipment than the reference method.

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