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Direct determination of chromium in gelatine by graphite furnace atomic absorption spectrophotometry

S. P. Quináia and J. A. Nóbrega*

Grupo de Química Analítica, Departamento de Química, Centro de Ciências Exatas e de Tecnologia, Universidade Federal de São Carlos, Caixa Postal 676, 13560-970 São Carlos, SP, Brazil

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Abstract

A fast and direct method for the determination of Cr in gelatine using graphite furnace atomic absorption spectrophotometry with Zeeman-effect background correction is described. No sample pre-treatment was necessary, minimizing the risk of contamination. The concentration of chromium in gelatine was directly evaluated applying the standard additions method from a metal spiked gelatine (0.5% m/v) dispersed in 1% v/v nitric acid solution. The heating program was implemented in 68 s. The estimated mean characteristic mass is 3.4 pg Cr for a 15 µl sample. The Cr values determined in gelatine with the proposed procedure are in close agreement with those obtained by employing an acid digestion procedure ($r = 0.9987$). © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Chromium; Gelatine; Graphite furnace; Electrothermal atomization

1. Introduction

It is well known that Cr(III) is essential and Cr(V1) is toxic. There are several procedures in the literature describing the redox speciation of Cr (Tsalev, 1995). In humans, Cr is involved in glucose and cholesterol metabolism, and its deficiency may cause neuropathy and encephalopathy (Anderson, 1988). Some foods, such as mushrooms, prunes, raisins, asparagus, and wine, contain Cr concentrations slightly higher than those found in most sources, but there are no known foods eaten that are notable sources of dietary Cr. Gelatine is mainly consumed by adolescents and may be a source of Cr considering the production process, which involves extraction of collagenous from beef skin material. The use of chrome-tanned leather residues can contaminate the product with chromium (Alleavitch et al., 1989). All food gelatines may contain trace amounts of various heavy metals and this should be carefully monitored and controlled within the limits of any applicable regulations. Considering the concentration range and the complexity of the sample, which is rich in organic compounds, such as carbohydrates and proteins, the determination of chromium in gelatines is not a trivial analytical task.

One of the modern analytical techniques that could be used for trace determination of Cr is inductively coupled plasma mass spectrometry (ICP–MS). However, low resolution ICP–MS's instruments are not completely suitable for Cr determination owing to the interferences caused by the isobaric compound $^{52}ArC^{+}$. These interferences cannot be entirely corrected using mathematical equations and require the total destruction of carbon compounds to avoid the argon carbide formation or a high resolution instrument. On the other hand, the complete destruction of carbon compounds is not easily performed and can need the use of a high pressure asher system (Krushevska et al., 1998). The use of closed microwave recipients can be troublesome due to the high volume of gases liberated by organic compounds.

Some procedures were proposed in the literature based on the use of acid mixtures and H_2O_2 for gelatine decomposition, but there is no clear information about the residual carbon content in the digested solution (Chung and Tsai, 1992; Chem et al., 1995; Zhang et al., 1996). This aspect was not critical because in these works flame atomic absorption spectrophotometry

*Corresponding author. Tel.: +55-16-260-8208; fax: +55-16-260-8350; e-mail: djan@zaz.com.br

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(FAAS) was employed for determining Ca, Mg, Fe, Cu, and Sn.

Flame atomic absorption spectrophotometry could be used for Cr determination in gelatines, but considering the low concentrations expected it is better to employ graphite furnace atomic absorption spectrophotometry (GFAAS). In addition to the good sensitivity, another advantage of the use of GFAAS is the possibility of the direct introduction of the gelatine suspension in the graphite tube, without any previous sample treatment. In spite of the complexity and the huge amount of organic components in gelatine samples, the direct analysis of gelatines by GFAAS is still a possibility to be investigated owing to the technological advancements of the instrumentation in this area. One previous work proposed the direct determination of Ge by Zeeman-GFAAS in gelatines, but the procedure involved the sample oxidation with a mixture of HNO_3 plus H_2O_2, thus the meaning of the so called direct determination is unclear (Chen and Chen, 1991).

The aim of this work was to develop a direct procedure for Cr determination by GFAAS. The objective is to establish a procedure with simple or no sample pretreatment before sample introduction into a graphite furnace. To reach this intent, samples were suspended in different media and the effects of each media on atomization processes were evaluated. The background signals with different heating cycles and the quantification procedure were also studied.

2. Experimental

2.1. Instrumentation

Measurements were made using a Varian Model 800 atomic absorption spectrophotometer equipped with a graphite furnace atomizer and autosampler (GTA 100). A Cr hollow cathode lamp was employed as the radiation source and the operating conditions are presented in Table 1. Pyrolytic coated graphite tubes (Part Number 63-100023-00) were used throughout the study. A volume of 15 µl gelatine suspension was directly dispensed into the graphite tube with the autosampler. Background correction was performed by Zeeman effect. Argon was used as the purge gas.

2.2. Reagents

All chemicals used in this study were of analytical-reagent grade (Merck). The glass and polypropylene apparatus were kept in 10% v/v HNO_3 solution for at least one night and then rinsed with deionized water before use. A 1000 mg l^{-1} Cr stock solution was prepared by dissolving metallic Cr in 1:1 v/v HCl under heating, and the obtained solution was properly diluted in deionized water. Solutions containing 1% v/v nitric acid and 1% v/v ammonium were prepared with deionized water. For the comparison procedure adopted, the gelatine acid digestion was carried out using HNO_3 and H_2O_2.

Table 1
Instrumental conditions and heating program

Lamp current.....7 mA	Wavelength.....357.9 nm
Slit width.....0.7 nm	Background correction.....Zeeman
Injection volume.....15 µl	Measurement mode.....peak area

Furnace program

Step	T (°C)	Time (s)	Flow gas (litre min^{-1})	Read
1	85	5	3	No
2	95	40	3	No
3	120	10	3	No
4	800	5	3	No
5	800	1	3	No
6	800	2	0	No
7	2400	1	0	Yes
8	2400	2	0	Yes
9	2500	2	3	No

2.3. Procedure

2.3.1. Sample dissolution and Cr atomization: effects of pH and sample volume

A mass of about 1 g of gelatine, accurately weighed, was suspended in three different media: 1% v/v nitric acid solution, 1% v/v ammonium solution, and deionized water. The suspensions were slightly heated at a temperature of 40°C during 5 min. After cooling to room temperature, the volume was made up to 100 ml in each one of these media. By action of an autosampler, a volume of 15 µl of the samples was introduced into a pyrolytic coated graphite tube. The effect of sample volume was studied from 10 to 40 µl. Peak area and peak height were measured and the quantification was performed using peak area values owing to better sensitivity and reproducibility.

2.3.2. Heating program: pyrolysis curves

For establishment of the maximum allowable pyrolysis temperature without Cr losses, pyrolysis curves were experimentally obtained in nitric acid medium. These curves were generated following a recommended procedure (Welz, 1985) by varying the temperature from 200 to 1800°C. A heating program without a pyrolysis step was also evaluated. The previously optimized atomization temperature was 2400°C. The furnace program developed is shown in Table 1.

2.3.3. Determination of Cr in gelatines

Two quantification procedures were employed to assess matrix effects: the first one using Cr reference

solutions prepared in HNO_3 solution and the other adopting the standard additions method (SAM).

For the development of the procedure, 10 gelatine samples were selected to provide variation of the matrix. Different trade marks of gelatine with and without flavor were used to evaluate possible effects caused by dyes and Cr contamination coming from raw materials in different industrial plants. All samples were obtained from local foodstores. All gelatines were dispersed in 1% v/v HNO_3 solution to a final concentration of 0.5% m/v.

The Cr values in gelatine were determined by the standard additions method. A 10 μg/l Cr reference solution prepared in HNO_3 was used to add Cr to gelatine suspensions to attain added concentrations of 2.0, 4.0, and 6.0 μg/l of Cr in 0.5% m/v gelatine. This procedure was programmed and performed by the autosampler.

2.3.4. Acid digestion procedure

For comparison purposes, an acid digestion procedure was implemented by adding 5 ml HNO_3 70% v/v to 0.5 g of gelatine samples. The samples were left overnight at room temperature, and after that 0.5 ml H_2O_2 was added to each solution followed by heating during about 30 min in a heating plate inside a fume hood for expelling the nitrogen oxides. After cooling at room temperature, the solutions were diluted to 100 ml with water.

2.3.5. Recommended procedure

A mass of 0.5 g of gelatine is suspended in 50 ml of 1% v/v HNO_3 solution. The suspension is heated at 40°C during 5 min. After cooling to room temperature, the clear suspension is transferred to a 100 ml volumetric flask and the volume is made up to the mark with 1% v/v HNO_3 solution. A volume of the sample suspension is transferred to the autosampler cup and one aliquot of 15 μl is automatically introduced into the pyrolytic coated graphite tube. The instrumental conditions and the heating program are shown in the Table 1. Quantification is performed using the standard additions method as described above.

3. Results and discussion

3.1. Sample dissolution and Cr atomization: effect of pH

As discussed in the Experimental section, gelatine samples were dispersed in acid, neutral and alkaline media. The main objective was to find a medium able to avoid the gelification of the sample and to disperse a mass of sample in a solvent volume compatible with the sensitivity of the GFAAS technique. All media evaluated attained these objectives showing that the gelatine gel formation is not dependent on the pH. A clear suspension was obtained in all cases. The next experiment investigated the media effects on the atomization process. To perform this preliminary study, a heating program previously established for the determination of Cr in urine samples was used (Quináia and Nóbrega, 1998). This program was chosen in these preliminary experiments taking into account that both samples, i.e. urine and gelatine, contain complex matrices and the direct analysis by GFAAS should be done by using a proper thermal program.

The peak areas were similar in neutral and alkaline media and 1 0% better in nitric acid medium (Fig. 1). This study was carried out in suspensions containing 1% m/v gelatine. In nitric acid medium, the peak profile was slightly more symmetric indicating a less complex atomization process. Taking into account both the better sensitivity and atomization process, all further measurements were made in nitric acid medium. The dispersions were prepared in a 0.5% m/v gelatine concentration since the repeatability was better in this medium. The relative standard deviations (RSD) were 2.3 and 4.7% ($n = 5$) for suspensions containing 0.5 and 1% m/v gelatine, respectively.

3.2. Heating program: pyrolysis curves and sample volume

The pyrolysis curves were obtained by introducing 15 μl of 0.5% m/v gelatine suspensions, two samples of gelatine were used, one with and another without flavor. Since the results obtained for gelatines with and without flavor are identical, only the atomic (AA) and background (BG) signals for the gelatine without flavor are shown in Fig. 2. The maximum pyrolysis temperature without Cr losses is 1600°C. Above this temperature Cr is totally volatilized. This is the behaviour expected even in the absence of the chemical modifiers owing to the refractory character of chromium and the possible formation of chromium carbide (Hoenig and Kersabiec, 1990). In spite of the direct introduction of a complex sample, the BG signal is low as depicted in Fig. 2. It can

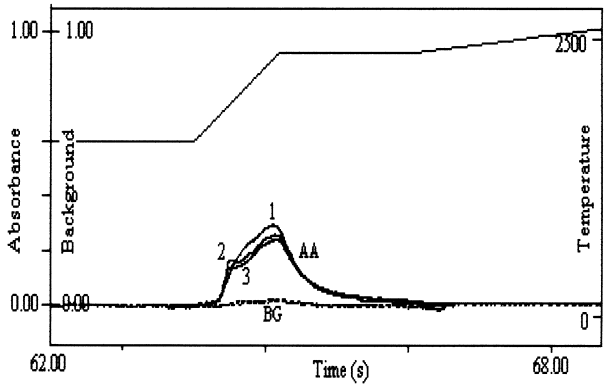

Fig. 1. Cr atomization signals in different media. 1. 1% m/v HNO_3 solution; 2. ammonium 1% m/v solution; 3. H_2O.

be seen that the BG signal is low even without using a pyrolysis step. The signals shown in Fig. 3 indicate the occurrence of a temporal separation between AA and BG signals when the pyrolysis step was excluded in the heating program. The most employed Cr resonance wavelength for measuring AA signals is 357.9 nm. The deuterium source continuum BG corrector has a low emission intensity at this wavelength, which makes difficult the use of this BG corrector for Cr measurements (Hoenig and Kersabiec, 1990). The observed temporal separation between AA and BG signals without a pyrolysis step allows the supposition that it could be possible to determine Cr directly in gelatine even without a BG corrector. In our case a heating program with an 8 s pyrolysis step at 800°C was adopted to completely eliminate the BG before the atomization step since it was used a Zeeman-GFAAS. This strategy was employed to avoid any interaction between vapour species during Cr atomization, and no losses of sensitivity or in the graphite tube lifetime happened. The minimum tube lifetime was 120 heating cycles and in most cases it was not observed a gradual decrease in sensitivity or repeatability during tube lifetime.

The effect of sample volume was studied from 10 to 40 µl. The main effect of sample volume was on the sensitivity, and this parameter can be chosen according to the Cr amount in the sample. However, with 40 µl the relative standard deviation was 3-fold higher than that obtained in lower volumes. For 10 and 35 µl the RSDs were 1 and 2.4%, respectively.

For a 15 µl sample suspension in nitric acid medium, the heating program took 68 s.

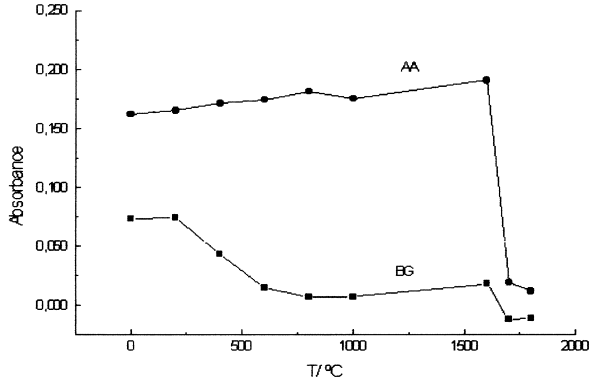

Fig. 2. Cr pyrolysis curve: atomic (AA) and background (BG) signals for a 0.5% m/v gelatine suspension (1% v/v HNO_3 solution).

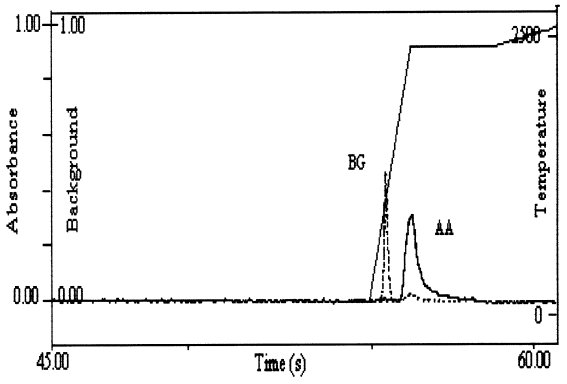

Fig. 3. Temporal separation between AA and BG signals without a pyrolysis step.

3.3. Determination of Cr in gelatine

After establishing the procedure to obtain the gelatine suspension and the conditions to atomize Cr in the graphite furnace, the next step was to evaluate the procedure that should be used for quantification of Cr in the samples. Two procedures were investigated: the first one using Cr reference solutions prepared in nitric acid medium and the other one employing the SAM. As expected, the Cr atomization was different in media with or without the matrix. The slopes of the reference curves were 0.055 and 0.026 in nitric acid and pure gelatine media, respectively. The slopes observed for gelatine suspensions using the SAM varied from 0.014 to 0.026 for gelatines with and without dyes, respectively. This means that there is a different behaviour in each medium and the use of the SAM is mandatory. The results obtained are shown in Table 2. Most results are low and probably there is no consequence related to public health. We do not know any relevant information about legislation related to Cr content in gelatine. The Food and Drug Administration in its world-wide web site only recommends that the raw material should be pure and metals free and the processing should follow acceptable industrial standards. Comparatively, only two data showed high Cr values (samples 1 and 4), and it was astonishing that one of these products is produced and sold in the USA (sample 4) and the other is produced and sold in Brazil as a 'natural' product (sample 1). The results were not validated using a certified reference material (CRM) since it seems that there is no CRM for metals in gelatine (IAEA, 1995).

Table 2
Determination of Cr in gelatines: proposed and acid digestion methods (mean values ± standard deviation; $n = 5$)

Gelatine sample	Concentration µg $Cr/g_{gelatine}$	
	Proposed procedure	Acid digestion procedure
1. Without flavor	21.9 ± 2.3	21.5 ± 0.8
2. Without flavor	1.47 ± 0.18	1.75 ± 0.43
3. Without flavor	1.77 ± 0.71	2.06 ± 0.34
4. Without flavor	4.88 ± 0.35	3.77 ± 0.23
5. Strawberry	0.33 ± 0.05	0.30 ± 0.04
6. Raspberry	0.55 ± 0.10	0.55 ± 0.06
7. Pineapple	0.49 ± 0.07	0.47 ± 0.05
8. Strawberry	0.38 ± 0.02	0.36 ± 0.04
9. Strawberry	0.84 ± 0.08	1.04 ± 0.16
10. Strawberry	0.69 ± 0.04	0.61 ± 0.04

The main advantage of the procedure developed is that it can be directly applied without any sample pre-treatment and the graphite tube lifetime was around 120 measurements, allowing the fast determination of chromium with a heating program implemented in only 68 s. The estimated mean characteristic mass was 3.4 pg of Cr and it is completely suitable considering the required sensitivity.

An acid digestion procedure was used for comparison of results. The solutions obtained by this procedure were yellow or pale-yellow depending on the Cr concentration in the sample. For one gelatine sample (sample 4) the resulting solution contained a fine white powder precipitated, which is indicative of an incomplete digestion. The results obtained for five replicates of each sample are shown in Table 2. Taking into account the mean results obtained by each method, the linear correlation coefficient calculated from these values is 0.9987 and the slope is 1.0079. Both parameters are indicative of the good agreement of the methods, showing that the proposed method is accurate and its use is advantageous owing to the direct introduction of the gelatine suspension into the graphite tube.

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References

Alleavitch, J, Turner, W. A. and Finch, C. A. (1989) In B. Elvers, S. Hawkins, M. Ravenscroft, J. F. Rounsaville, & G. Schulz, (Eds.), *Ullmann's encyclopedia of industrial chemistry*. Weinheim: VCH Publishers.

Anderson, R. A. (1988) In K. T. Smith (Ed.), *Trace minerals in foods*. New York: Marcel Dekker.

Chem, S. Y., Zhang, M., Lim, S. Q. and Cheng, L.Determination of trace iron and copper in gelatine by flame atomic-absorption spectrophotometry with a flow-injection extraction system. *Guangpuxue-Yu-Guangpu-Fenxi, 15*, 85–90.

Chen, Z. and Chen, Y. (1991) Direct determination of ultra-trace germanium by Zeeman graphite-furnace atomic-absorption. *Fenxi-Huaxue, 19*, 1405–1407.

Chung, S. W. and Tsai, W. C. (1992) Atomic-absorption spectrometric determination of heavy metals in foodstuffs using a simple digester. *Atomic Spectroscopy, 13*, 185–189.

Hoenig, M. and Kersabiec, A.-M. (1990) *L 'atomisation électrothermique en spectrométrie d'absorption atomique*. Paris: Masson.

International Atomic Energy Agency (1995). *Survey of reference materials*. Vol. 1: *Biological and environmental reference materials for trace elements, nuclides and microcontaminants*. IAEA-TEC-DOC-854, Vienna.

Krushevska, A., Waheed, S., Nóbrega, J. A., Amarisiriwardena, D. and Barnes, R. M. (1998). Reducing polyatomic interferences in the ICP-MS determination of chromium and vanadium in biofluids and tissues. *Applied Spectroscopy* **52**, 205–211.

Quináia, S. P. and Nóbrega, J. A. (in press) Determination of chromium in urine by graphite furnace atomic absorption spectrophotometry. *Fresenius Journal of Analytical Chemistry*.

Tsalev, D. L. (1995). *Atomic absorption spectrometry in occupational and environmental health practice* (Vol. III). Boca Raton: CRC Press.

Zhang, M., Chen, S. Y., Liu, S. Q. and Cheng, L.FAAS determination of calcium and magnesium in gelatine using the FIA online automatic dilution technique. *Lihua-Jianyan, -Huaxue-Fence, 32*, 82–83.

Welz, B. (1985). *Atomic absorption spectrometry* (2nd ed.). Weinheim: VCH Publishers.