

# OPTIMIZATION OF A SEQUENTIAL EXTRACTION SCHEME FOR SPECIATION OF METALS IN FINE URBAN PARTICLES

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A sequential extraction procedure fractionated in four steps was optimized with the purpose of improving a previous scheme for determining the chemical forms of metals in fine airborne particles. The optimization was tested on synthetic samples and then confirmed on real samples of fine particles, optimizing the type of reagent, its concentration, pH, temperature and extraction time. Synthetic samples were prepared in the laboratory with compounds of reagent grade. The analytical method applied consisted of extracting the metals from each sample in a rotator with each type of reagent under different conditions of the parameters and then centrifuging before measuring by ICP-AES. The improved and previous scheme was checked on real samples and the differences were significant in the percentages of the soluble chemical forms in the improved scheme (5.2% in front to 22.0% for Pb, 27.0% in front to 50.0% for Cd and 32.4% in front to 9.0% for Ni) from 55.6, 0.23 and 1.48 ng m<sup>-3</sup> of total metal concentrations respectively.

*Keywords:* Chemical speciation; Metals; Airborne; Optimization; Fine particles; Urban pollution

## INTRODUCTION

Urban populations are exposed to the metals in airborne particles. When the metal concentration is high, it can pose serious risk to human health. However, determination of metal levels is usually limited to determining

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the total metal concentration. These measures provide us information neither on the chemical forms. Therefore, particulate standards based on total suspended particles (TSP) alone are insufficient [1]. Knowledge of the chemical speciation of the metals is vital in understanding the effects on the public health [2,3].

Current bibliography on chemical speciation of sediments, soils, sludges and biological systems is wider than those on atmospheric particles [4]. Many authors use extraction schemes based on the Tessier's scheme [5,6]. A research in which this scheme was applied on atmospheric particles (with several modifications) was performed in Barcelona (Spain) [7]. The BCR®'s scheme (Bureau of Certified Reference) was published later on [8] and it was also applied to sediments [9] and sludges [10] and introduced important changes. In 1993–1995 we studied the fractionation of 10 metals in the aerosols of Seville using the scheme of Barcelona [11] (see Table I).

A first objective of this present work is to optimize a speciation scheme for fine particles in a city influenced mainly by traffic, but also by earth crustal particles mixed with particles from industrial sources [12]. There is a great difference between a matrix of sediments and that of airborne particles. Sediments contain high contents of organic matter, iron and manganese oxides and silicates. Atmospheric particles generally also have important levels of organic matter. Particles contain other oxidised metals as geological material. Also, particles contain elemental carbon, sulphate, nitrate, chloride and ammonium [13]. For this reason, some stages of the Tessier's scheme is modified for particles [7,14,15]. Both Tessier's modified

TABLE I Speciation scheme from the Tessier's modified scheme according to Obiols *et al.* (1986)

<i>Metallic fraction</i>	<i>Experimental conditions</i>
1 Soluble and exchangeable	25 mL of 1% NaCl mechanical agitation during 60 min at room temperature
2 Carbonates, oxides and reducible	25 mL of 0.04 M NH <sub>2</sub> OHHCl in 25% HAcO 1 h at 95°C, agitating occasionally
3 Bound to organic matter, oxidisable and sulphidic	25 mL of 0.02 M HNO <sub>3</sub> + 10 mL 30% H <sub>2</sub> O <sub>2</sub> 90 minutes at 85°C + 3 mL 30% H <sub>2</sub> O <sub>2</sub> 1 hour at 85°C + 5 mL of 3.2 M NH <sub>4</sub> AcO in 20% HNO <sub>3</sub> continuous agitation 30 min at room temperature
4 Residual	5 mL of conc. HNO <sub>3</sub> + 2 mL of conc. HCl + 20 mL H <sub>2</sub> O 90 min at 95°C, agitating occasionally

scheme and BCR<sup>®</sup> were tested by us in 1996–1998, in order to find out which experimental conditions were the most appropriate to particles [16].

A second objective is to determine the chemical forms that can be available to the humans. The metal bioavailability was already studied by us in a previous work under the aspect of the physical speciation [17]. In this work the smallest particles were the more bioavailable and there were toxic metals that trend to accumulate in these particles (Ni, Pb, Cd). Therefore, the optimization of the chemical speciation should be carried out in the fine particles, that is to say, below 1  $\mu\text{m}$  (ISO 7708, 1995) [18].

The speciation scheme were optimized in order to determine the chemical forms in which the metals present in the particulate matter can become to the respiratory tract [19]. With this aim, the experimental conditions of the scheme were designed in search of similar conditions into the lung and it is necessary to simulate the physical and chemical conditions found in the respiratory tract (redox potential, temperature and pH mainly).

The optimization uses synthetic samples [20–22] applied to atmospheric particles [23,24]. The samples are prepared in the laboratory mixing chemicals of reagent grade [25,26] and the metallic concentrations are therefore, known. Optimization is done using the synthetic samples separately and, in order to confirm the optimized results, the procedure is repeated on real samples of particles.

## EXPERIMENTAL

### Procedure for the Optimization of Scheme

The procedure consisted of applying different experimental conditions to these solid mixtures, modifying in these different tests parameters such as the type of reagent, its concentration, pH, temperature and extraction time. The optimal condition of the parameter was selected to achieve these known metal concentrations. All the parameters were optimized by plotting the graphs of the different tests for each metal representing the recoveries with respect to the theoretical concentration (the 100%) and then evaluating the optimal value of all the metals as a whole.

When this procedure is applied to real samples, whose metallic concentration is unknown, the recovery of 100% of each test is determined taking the optimal value of the parameter as the greater metal concentration of the four determinations corresponding to the maximum of the curve for the whole of all the metals, that is the maximum concentration of metal extracted for the

most of the 11 elements (although several metals are extracted better at other value of the parameter). Then the corresponding percentages of the other values of the parameter of each test are calculated and then averaged for the four determinations. It is because of this that the averaged values are not necessarily 100.0% accurate.

To optimize the 5 synthetic samples corresponding to the four fractions, 13 tests were needed. The last parameter optimized, the extraction time, was always done in real samples for obtaining an optimal time more in agreement with the reality, and all the parameters optimized in the third fraction was also done in real samples, after the two first fractions were optimized and applied on the same samples previously. Each test consisted of modifying the value of the corresponding parameter by preparing 8 different solutions (except only 3 types for the residual fraction). Therefore, a total of 99 different solutions were measured by quadruplicate and a total of  $99 \times 11 \times 4 \frac{1}{4}$  4356 different concentrations for the 11 elements were determined. When these optimal conditions were obtained, they were applied on the real samples (except the time of extraction and all the parameters of the third fraction already done) and, consequently, they constituted a total of  $7 \text{ tests} \times 8 \times 11 \times 4 \frac{1}{4}$  2464 different concentrations. If these results agree with those obtained with the synthetic samples, this means that the design of the synthetic samples has been valid for the experimentation and this fact gives validity to the resulting experimental conditions.

Additionally this large amount of data ( $4356 + 2464 \frac{1}{4}$  6820) assure the quality of the optimization.

When the four fractions have been optimized separately, all the optimized conditions were tested together on the synthetic sample 5 ('total'), and in this way to obtain the final improved scheme. Also, the four experimental conditions of the four fractions were tested on the synthetic samples not corresponding to each fraction, for instance, applying the conditions corresponding to the first fraction to the synthetic samples corresponding to the second, third and fourth fraction, checking that the metals extracted represent only a negligible percentage. In this way we can verify for example that the metals that should appear in the residual fraction donot appear in previous fractions.

Finally, the scheme was applied on 10 real samples collected in 10 sampling sites of Seville for corroborating the results with the improved scheme and also it was applied on the same samples with the previous scheme of our previous work [11] for studying the differences and their significances.

### Preparation of Synthetic Samples

Five mixtures ‘solubles’ (chlorides, sulphates and nitrates) to optimize the first fraction, ‘oxides’ and ‘carbonates’ (carbonates and hydroxycarbonates) to optimize the second fraction, ‘residuals’ (pure metals) to optimize the fourth fraction and one with all the previous compounds together (‘total’) were prepared. Hygroscopic substances were dried in a dessicator for 48 h to avoid the thermal decomposition or the melt. Besides the dessicator, care was taken in handling several reagents in order to avoid toxicological problems and rigorous reactions with the water of the moisture, and therefore, a laminar flux cabinet was also used here. For the third fraction, the optimization was carried out directly on samples of airborne particles, as it is explained below.

Synthetic samples were prepared as follows: 1 g of each compound was carefully and accurately weighed and put into polypropylene flask with a thread stopper. After having weighed all the compounds, the resulting mixture was multi-coloured, due to the different colours of each reagent.

The flasks of each mixtures were, then, dried again in the dessicator for another 48 h, to eliminate the possible incorporation of moisture during the process of weighing.

Finally, the stopper was put back in the tube and this was then mechanically shaken for 48 h in the vertical vibrator, after which the initial colours turned to monochrome and the particle size became fine and homogenous. This homogeneity was corroborated by optical microscopy and it was also verified microscopically that this time for 48 h was sufficient for obtaining a size equal to the fine particles.

In order to obtain metal concentrations from each synthetic sample, these were extracted with acid digestion on 30 mg of sample accurately weighed in polypropylene tubes with a mixture of nitric and hydrochloric acids (3 : 1) using a water-bath at 95°C for 90 min. This determination was performed in quadruplicate. Then the four 100 mL flasks were measured by ICP-AES. The results were compared with the theoretical values and they agreed (see Table II).

### Suspended Particle Sampling

Fine airborne particles were collected in quartz filters ( $20.3 \times 25.4 \text{ cm}^2$ ) from WHATMAN (QM/A) with a sampler (MCV, Model CAV-A/HF) equipped with a cascade impactor (MCV, Model IC/CAV). Particles enter the impactor at a flow rate of  $68 \text{ m}^3 \text{ h}^{-1}$ . The impactor effectively separates

TABLE II Recoveries in percentages with respect to the theoretical values for the 11 elements in each synthetic sample

<i>Synthetic sample</i>	<i>Percentage of theoretical value(%)</i>											
	<i>Ca</i>	<i>Fe</i>	<i>Mg</i>	<i>Pb</i>	<i>Cu</i>	<i>Mn</i>	<i>V</i>	<i>Ti</i>	<i>Ni</i>	<i>Co</i>	<i>Cd</i>	<i>Mean</i>
1 Solubles	81.4 ± 12.3	98.2 ± 10.6	90.3 ± 3.5	105.5 ± 13.2	99.6 ± 4.9	100.3 ± 8.3	91.1 ± 11.1	97.6 ± 17.6	103.1 ± 8.4	103.4 ± 4.0	106.3 ± 13.3	97.9 ± 35.2
2 Carbonates	104.4 ± 6.0	–	95.7 ± 9.1	105.2 ± 15.5	101.1 ± 11.5	94.0 ± 5.2	–	–	100.6 ± 15.5	94.9 ± 8.0	91.5 ± 3.0	98.4 ± 28.8
3 Oxides	92.0 ± 6.3	11.4 ± 5.7	94.4 ± 7.8	103.0 ± 7.2	101.1 ± 4.8	82.1 ± 6.3	112.3 ± 12.5	17.1 ± 3.1	95.0 ± 7.3	99.5 ± 7.0	25.8 ± 5.2	75.8 ± 23.3
4 Pure metals	96.0 ± 3.2	91.4 ± 4.8	99.8 ± 3.1	99.4 ± 3.2	95.0 ± 2.4	97.1 ± 7.4	84.2 ± 4.3	2.5 ± 2.0	96.9 ± 5.7	89.7 ± 18.0	4.8 ± 2.5	77.9 ± 22.3
5 Total	82.3 ± 14.1	68.9 ± 11.9	95.4 ± 9.4	96.7 ± 10.6	94.0 ± 12.2	90.6 ± 4.7	94.2 ± 19.5	43.6 ± 15.3	95.1 ± 18.3	97.6 ± 8.9	70.6 ± 17.1	84.5 ± 42.4

the particles in six stages and the last filter collects particles smaller than 0.61 mm [17].

13 samples of particles needful for the optimization were collected in Reina Mercedes, a representative area of Seville, Spain [12,27]. This sampling station represents traffic and industrial emissions. These samples were collected until we obtained the necessary weight of particles, with sampling periods of 96 h collecting about 6500 m<sup>3</sup> of air and 600 mg of particles. The 10 samples for testing the improved and previous schemes were collected from our network of sampling stations [11].

#### Reagents and Apparatus

Care was taken in handling the samples in order to avoid contamination problems within a vertical laminar airflow cabinet with a HEPA filter from INDELAB (Model IDL-48V). Water bath was from JULABO (Model SW-20C). Centrifuge was from SIGMA (Model 3-15). Reagents and standard solutions for metals were from MERCK. Vertical vibrator for mixing the chemical reagents was from SELECTA (Vibromatic Model 384). Milli-Q-grade water was from WATERS-MILLIPORE (Model Plus). The sample extractions were analysed for 11 elements (Mg, Ca, Ti, V, Mn, Fe, Co, Ni, Cu, Cd and Pb) by inductively coupled plasma atomic emission spectrometry (ICP-AES) using a Fisons-ARL 3410 instrument. One minitorch consumes argon gas at a radio frequency power of 650 W, consequently, it is capable of consuming a few millilitres of sample at a flow of 2.3 mL min<sup>-1</sup>. This fact allows that the metals usually measured by FAAS and GFAAS in 50-mL flasks in our previous works can also now be measured by ICP-AES with only 15 mL.

#### Methodology for Chemical Analysis

Optimization tests were carried out in polypropylene centrifugal tubes by adding 30 mg of each mixture accurately weighed and subsequently applying the corresponding experimental conditions. 15 mL of each reagent was added. Extractions were carried out in a rotator at 50 rpm and at ambient temperature. Centrifugation was performed at 5000 rpm for 10 min. Finally, the tube was left to decant by pouring the liquid into 100 mL flasks for measuring by ICP-AES.

The matrix effect due to the particle and filter matrix was studied through the standard addition technique in the four speciation fractions.

The standard addition verified that this matrix effect was null. Regarding the interference matrix due to the reagents, the calibration curves have been obtained with the same matrix as each one of the four speciation fractions.

After sampling, each filter was dried and weighed [17] and then cut into 8 eighths, each of which was then placed in a polypropylene centrifuge tube. The tests were carried out on each eighth with the same procedure as that for synthetic samples. The only difference was the follows: the different tests of the second fraction and subsequents were applied to the residue of the previous fraction already optimized. For these samples, a set of unexposed filters was analysed using the same procedure in each fraction of the scheme. The mean unexposed filter value was subtracted from each real sample. For testing the scheme over the 'total' synthetic sample and over the real samples the procedure was to apply the four fractions in a continuous sequence.

## RESULTS AND DISCUSSION

### Optimization of the First Fraction of the Scheme: Soluble and Exchangeable

In order to optimize the type of reagent we know that different schemes use water [15], magnesium chloride or sodium acetate [5], sodium chloride [7], acetic acid [8], barium chloride [28]. Tessier's modified scheme uses sodium chloride and BCR® scheme [29] uses acetic acid. Therefore, both sodium chloride and acetic acid were tested.

For optimizing the concentration of sodium chloride, 8 solutions were prepared from 0.0 (water) to 3.5%. For acetic acid the 8 solutions prepared were from 0.01 to 0.15 M.

15 mL of solution was added to each polypropylene tube containing the synthetic sample 1. The tubes were closed and rotated at 50 rpm for 5 h. The sodium chloride extracts the 100% and recoveries are independent from the concentration and water (NaCl 0.0%) is the selected concentration. Some schemes have also found water as the best extractant of soluble metals [15,30]. The two tests were repeated on two real samples with the same results (see Fig. 1a).

For optimizing the pH, we keep in mind that SO<sub>2</sub> level in Seville is low [31] and our measures made in rainwater through the time have an average value of pH 7.3. Moreover, pH of normal lung is 7.40, which is



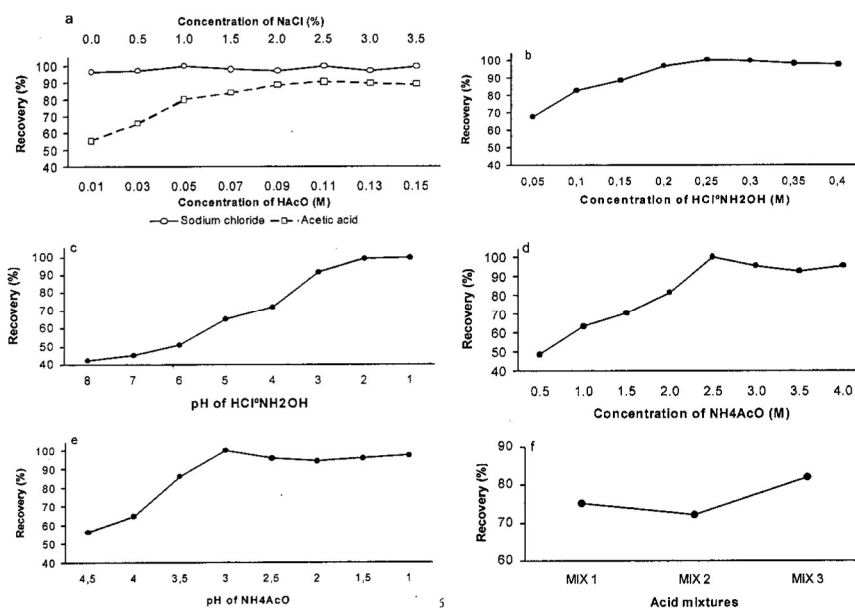


FIGURE 1 (a) Extraction of soluble and exchangeable metals from a real sample varying the concentration of sodium chloride and acetic acid. (b) Extraction of oxides, carbonates and reducible metals from a real sample varying the concentration of hydroxylamine chloride. (c) Extraction of oxides, carbonates and reducible metals from a real sample varying the pH of hydroxylamine chloride. (d) Extraction of bound to organic matter, reducible and sulphidic metals from a real sample varying the concentration of ammonium acetate. (e) Extraction of bound to organic matter, reducible and sulphidic metals from a real sample varying the pH of ammonium acetate. (f) Extraction of residual metals from a real sample with the three acid mixtures.

the physiological pH of the human body. If the pH of the lung mucosa changes, even below one unit, acid-based disorders can be caused, as a respiratory acidosis. Then, we will use a pH 7.4 for the water.

In order to optimize the time, 15 mL of water at pH 7.4 was added to 8 tubes containing an eighth of a new real sample. The tubes were, then quickly put into the rotator and one tube was taken out every 30 min. Then the results (see Table III) indicate that the very best time of extraction is 3 h.

#### Optimization of the Second Fraction of the Scheme: Oxides, Carbonates and Reducible

For selecting the type of reagent it is necessary to keep in mind that many speciation schemes commonly use hydroxylamine chloride in front of

TABLE III Variation of the extraction of the 11 elements with the time parameter

Elements	Percentage of extraction (%)										Mean	
	Ca	Fe	Mg	Pb	Cu	Mn	V	Ti	Ni	Co		Cd
Time (min)	Extraction of soluble and exchangeable metals											
30	100.0	76.1	81.4	77.3	86.1	82.2	73.3	76.2	68.8	78.8	64.4	78.6
60	98.3	87.0	84.6	76.0	89.6	85.8	79.1	89.1	84.1	81.5	66.7	83.8
90	99.1	92.4	86.6	80.0	92.8	90.6	76.7	91.3	79.1	79.9	72.2	85.5
120	97.6	95.3	88.6	86.7	95.1	96.9	84.7	92.3	89.0	91.7	77.8	90.5
150	99.4	92.4	95.7	92.0	97.5	98.6	93.3	93.6	94.0	95.8	88.9	94.7
180	98.9	99.7	100.1	100.0	99.8	100.3	100.0	99.8	100.2	100.0	100.0	99.9
210	98.3	96.7	95.7	96.0	98.6	98.6	96.7	97.2	89.0	97.8	96.6	96.5
240	99.8	97.8	98.7	98.0	99.8	99.5	98.7	98.6	93.0	98.8	94.4	97.9
Time (h)	Extraction of carbonates, oxides and red oxide metals											
1	29.0	25.1	24.5	19.5	20.5	23.1	15.6	24.6	27.2	26.0	30.1	24.1
2	32.6	46.2	31.2	69.2	33.3	30.1	19.9	49.8	68.5	32.2	31.2	40.4
3	45.8	55.4	42.1	72.3	45.1	43.3	39.8	60.5	72.6	64.5	44.4	53.3
4	70.0	65.6	66.7	95.1	60.9	54.2	60.2	70.8	82.5	81.2	58.3	69.6
5	100.4	100.5	100.2	100.4	100.4	99.9	100.0	100.0	99.8	100.5	99.9	100.2
6	88.0	100.0	100.0	97.0	99.0	100.0	98.1	100.0	100.0	98.2	86.0	96.9
7	82.9	96.4	100.0	100.0	98.2	94.6	97.1	97.9	100.0	93.1	74.0	94.0
8	98.0	96.2	95.2	95.2	95.1	96.2	98.8	97.2	95.2	94.2	98.3	96.3
Time (min)	Extraction of bound to organic matter, oxidable and sulfidic metals											
15	100.0	87.6	89.4	97.0	98.2	97.6	85.2	88.9	87.5	89.7	84.5	91.4
30	98.3	97.8	94.8	97.6	98.6	98.5	88.6	95.5	94.8	95.1	95.4	95.9
45	100.0	94.2	99.8	100.0	98.2	96.0	89.6	92.3	99.1	99.7	99.2	97.1
60	99.7	99.3	99.8	98.7	99.5	99.6	90.5	99.5	98.2	97.1	99.6	98.3
75	100.2	99.7	100.1	97.8	99.7	100.0	96.2	100.2	98.7	99.9	99.8	99.3
90	100.0	99.8	99.7	99.9	99.7	99.8	98.9	99.0	100.0	100.0	100.3	99.7
105	99.3	99.9	99.5	98.7	99.9	99.6	99.8	99.3	98.7	98.7	99.2	99.3
120	99.9	98.7	100.3	99.8	100.1	99.6	99.9	98.6	98.9	99.9	99.2	99.5
Time (h)	Extraction of residual metals											
1	92.0	90.5	93.0	92.1	90.5	91.5	93.2	89.5	90.1	95.6	93.5	92.0
2	98.7	95.2	98.8	95.2	96.5	96.1	94.6	89.9	94.0	94.2	97.8	95.5
3	96.2	100.0	98.6	98.5	97.5	99.2	96.4	92.0	99.5	99.1	95.2	97.5
4	100.4	99.8	99.5	100.3	99.9	100.1	97.6	98.9	99.8	100.3	99.9	99.7
5	100.0	96.7	95.2	100.0	95.1	100.0	98.9	98.3	100.0	95.8	100.0	98.2
6	99.0	100.0	100.0	99.6	100.0	99.3	99.5	98.1	97.9	96.5	98.7	99.0
7	99.8	99.7	99.8	100.0	100.3	100.2	96.2	99.1	100.0	99.9	99.9	99.5
8	99.8	97.2	98.7	100.0	97.2	100.0	97.6	97.1	98.0	97.0	96.2	98.1

others such as sodium pyrophosphate [14] or sodium dithionate-citrate [32] and, therefore, this reagent will be the selected one for this fraction. In this case we prepared 8 solutions of reagent from 0.05 M to 0.4 M applied to the synthetic fractions 2 and 3. The test was repeated on a new real sample (see Fig. 1b). The optimal concentration was observed at 0.25 M.

The pH commonly used is pH 2 in many schemes, therefore, the tests were carried out by preparing 8 solutions of hydroxylamine chloride at 0.25 M from pH 8 to pH 1 applied to the synthetic fractions 2 and 3.

The results are that the best conditions for the extractions were at pH 2. The experiment repeated on a real sample obtained the same result (see Fig. 1c).

In order to optimize the time of extraction, in this case the tubes were taken out every hour. A new filter of particles was used. The results (see Table III) showed that the optimal time was 5 h.

#### Optimization of the Third Fraction of the Scheme: Metal Bound to Organic Matter, Oxidisable and Sulphidic

In this fraction we tried to prepare humus containing metals but the results were not satisfactory and the third fraction was tested directly on real samples. Firstly, the organic matter of the particles is digested with hydrogen peroxide and then the metals liberated are quickly stabilized with ammonium acetate. The initial volume of hydrogen peroxide should become almost dry and the time required was 5 h at 95°C.

For optimizing the concentration, 8 solutions of ammonium acetate were prepared from 0.5 to 4.0 M. The optimal concentration was 2.5 M (see Fig. 1d). And for the pH, 8 solutions of reagent were prepared from pH 4.5 to 1.0. The optimal pH is 3.0 (see Fig. 1e).

The time was optimized by taking the tubes every 15 min. It can be seen (see Table III) that the percentage of extraction was independent from time and 90 min was sufficient.

#### Optimization of the Fourth Fraction of the Scheme: Residual

Tessier's original scheme uses an acid digestion with perchloric and hydrofluoric acids, but hydrofluoric acid cannot be used with fibreglass or quartz filters [12,27]. The procedure carried out in our studies [11,17] used perchloric and nitric acids, and the Tessier's modified scheme uses nitric and hydrochloric acids [7]. So, the three different acid mixtures nitric–hydrochloric (MIX1), nitric–perchloric (MIX2) and nitric–hydrochloric–perchloric (MIX3) in the proportions 5 : 2, 5 : 1 and 6 : 2 : 5, respectively, were tested on the synthetic fraction 4 and real samples.

15 mL of mixture were added to the 'residual' fraction. In both synthetic and real samples the three mixtures provide very good recovery rates, but the best is the triacid mixture (see Fig. 1f). The extraction time was evaluated with a new real sample, taking out the tubes every hour. 5 h proved to be sufficient time (see Table III).

### Final Speciation Scheme: Comparison with the Previous Scheme

Finally, the scheme was tested on the 'total' mixture obtaining the expected positive results. The resulting final scheme is shown in Table IV. In brief, the main differences of the new scheme regarding the previous one can be summarised as follows:

1. Water is used instead of sodium chloride. The soluble metallic species can be extracted. Also, sodium metallic species can be measured, although quartz or membrane filters would be needed.
2. The optimized concentration of the hydroxylamine chloride is different. The extraction is carried out at ambient temperature and in this way the extraction of the bioavailable chemical forms is more truthful.
3. pH and concentration of ammonium acetate were also different.

The final scheme was also tested on 10 particle samples collected in Seville and both the Tessier's modified scheme and the improved scheme were applied on these same samples with the aim of comparing the results. The concentration values and percentages of the chemical forms are shown in Table V. These concentrations were different in many metals. The more important differences are in the two first fractions. This change affects notably some metals like Cd, Mn, Co, Ni and especially Pb in the first fraction and the metals Fe, Pb, Cd and mainly Cu in the second fraction. The new experimental conditions of the two first fractions in the new proposed scheme are less aggressive than those of the Tessier's modified scheme; therefore, it is expected that the percentages of several metals in

TABLE IV Speciation scheme proposed according to Ferná ndez Espinosa *et al.* (1998)

<i>Metallic fraction</i>	<i>Reagent</i>	<i>Experimental conditions</i>
1 Soluble and exchangeable	15 mL H <sub>2</sub> O (pH =7.4)	3 h at room temperature shaker agitation
2 Carbonates, oxides and reducible	10 mL NH <sub>2</sub> OHClH 0.25 M at pH = 2.0	5 h at room temperature shaker agitation
3 Bound to organic matter, oxidisable and sulphidic	7.5 mL H <sub>2</sub> O <sub>2</sub> 30%	at 95°C until near dryness shaker agitation
	+	+
	7.5 mL H <sub>2</sub> O <sub>2</sub> 30%	at 95°C until near dryness shaker agitation
4 Residual	+	+
	15 mL NH <sub>4</sub> AcO 2.5 M at pH = 3.0	90 min. at room temperature shaker agitation
	10 mL (HNO <sub>3</sub> :HCl:HClO <sub>4</sub> ) (6 : 2 : 5)	5 h at 95°C shaker agitation

TABLE V Results of concentrations in  $\text{ng m}^{-3}$  and percentages of the chemical forms of the 10 samples analysed by the speciation scheme proposed according to Ferná ndez Espinosa *et al.* (1998) and the Tessier's modified scheme according to Obiols *et al.* (1986)

Fraction	Proposed scheme ( $\text{ng m}^{-3}$ )										
	<i>Ca</i>	<i>Fe</i>	<i>Mg</i>	<i>Pb</i>	<i>Cu</i>	<i>Mn</i>	<i>V</i>	<i>Ti</i>	<i>Ni</i>	<i>Co</i>	<i>Cd</i>
Metal											
Soluble and exchangeable	197	15.2	21.9	2.9	2.57	1.69	2.31	0.18	0.48	0.179	0.062
Carbonates oxides and reducible	366	61.3	24.6	16.7	1.54	1.23	1.24	0.08	0.41	0.123	0.042
Bound to organic matter, oxidable and sulphidic	225	75.6	112.3	22.5	5.62	2.51	0.75	1.82	0.42	0.063	0.076
Residual	15	150.3	51.2	16.8	2.22	0.78	0.69	1.57	0.17	0.102	0.05
Sum	803	302.4	210	55.6	11.95	6.21	4.99	3.65	1.48	0.467	0.23
Fraction	Percentages										
Soluble and exchangeable	24.5	5.0	10.4	5.2	21.5	27.2	46.3	4.9	32.4	38.3	27.0
Carbonates oxides and reducible	45.6	20.3	11.7	30.0	12.9	19.8	24.8	2.2	27.7	26.3	18.3
Bound to organic matter, oxidable and sulphidic	28.0	25.0	53.5	40.5	47.0	40.4	15.0	49.9	28.4	13.5	33.0
Residual	1.9	49.7	24.4	30.2	18.6	12.6	13.8	43.0	11.5	21.8	21.7
Fraction	Tessier's modified scheme ( $\text{ng m}^{-3}$ )										
Soluble and exchangeable	331	8.4	32.6	12.0	2.08	2.06	2.16	0.12	0.11	0.158	0.055
Carbonates oxides and reducible	374	108.6	41.9	29.8	4.44	1.88	1.68	0.58	0.10	0.107	0.017
Bound to organic matter, oxidable and sulphidic	89	45.7	58.3	10.8	2.25	0.88	0.47	0.78	0.80	0.028	0.016
Residual	95	105.0	24.0	1.9	1.14	0.48	0.48	0.15	0.21	0.016	0.022
Sum	889	267.7	156.8	54.50	9.91	5.30	4.80	1.63	1.22	0.310	0.110
Fraction	Percentages										
Soluble and exchangeable	37.2	3.1	20.8	22.0	21.0	38.9	45.0	7.4	9.0	51.0	50.0
Carbonates oxides and reducible	42.1	40.6	26.7	54.7	44.8	35.5	35.0	35.6	8.2	34.5	15.5
Bound to organic matter, oxidable and sulphidic	10.0	17.1	37.2	19.8	22.7	16.6	9.8	47.9	65.6	9.0	14.5
Residual	10.7	39.2	15.3	3.5	11.5	9.1	10.0	9.2	17.2	5.2	20.0

(After the results and discussion section: final speciation scheme).

these fractions decrease. Pb and Cd have lower percentages in the soluble and exchangeable chemical forms in the proposed scheme (5% and 27% in front of 22% and 50% respectively). However, Ni chemical species increases its percentage in the soluble fraction (32% in front of 9%). In the second fraction, the metals Cu and Fe have lower percentages (13% and 5% in front of 45% and 41% respectively). In brief, the decreases in the bioavailable fractions are encouraging because cadmium and lead, objects of study by the European Community, are very toxic. However, the same is not expected for nickel because of the increase in the soluble fraction.

## CONCLUSIONS

Optimization of the speciation scheme has enabled us to develop a new improved scheme for airborne particles. This scheme improves the Tessier's modified scheme. The use of pure water with a pH 7.4 in the first fraction makes it possible to carry out the extraction under similar conditions to those existing in the lungs. In order to extract the reducible metals, many speciation schemes use high temperatures to dissolve insoluble oxides. However, these extreme temperatures do not exist in the respiratory tract. Furthermore, pulmonary conditions have a reducing effect that is characteristic of the organic matter by which the pulmonary mucosa is formed. It is well known in medicine that an oxidizing environment in the lungs may cause serious damage [33]. This is why the optimization was carried out at ambient temperature, using hydroxylamine chloride. To extract the metals from the third fraction, it was applied consecutively on the two first fractions already optimized on real samples and it was optimized by the parameters for the ammonium acetate after oxidizing the organic matter with hydrogen peroxide. When optimizing the fourth fraction the best result was obtained with the nitric-perchloric-hydrochloric acid mixture 6 : 5 : 2.

We consider that this speciation for metals in suspended particles shows clearly the distribution of the different chemical forms and provides us with very valuable information on the chemical toxicity of metals in the human body. The results obtained suggest new working conditions for metals in airborne particles as well as some conclusions on the bioavailability of metals in respirable particles.

### *Acknowledgment*

We would like to thank the 'Consejería de Medio Ambiente de la Junta de Andalucía' for their financial assistance to carry out this research project in our Department, and for the grant awarded to the corresponding author with which he finished his Doctoral Thesis in 1998.

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