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The Effect of Acid Digestion on the Recoveries of Trace Elements: Recommended Policies for the Elimination of Losses

Güler SOMER*, Arzu NAKIŞCI ÜNLÜ
Gazi University, Faculty of Arts and Science, Department of Chemistry, 06500 Ankara-TURKEY
e-mail: gsomer@gazi.edu.tr

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Digestion of biological materials is very important for trace element determinations. Although the microwave technique is mostly used, there are cases where open wet digestion is needed. This study examined the optimum conditions for the minimum loss of trace elements during the wet digestion of a sample. The effect of acid composition and digestion time for about 21 ions was investigated. The trace element concentrations were determined by differential pulse polarography. For the digestion procedure, a $HClO_4$-HNO_3 mixture was used for the first step and, after its evaporation (60 min), HCl was added as the second step, since in some cases it was necessary. The recoveries were high for ions like Zn, Cu, Ti(IV), Cd, Fe, Mn(II), Co(II), V(III) and Mo(VI) after digestion (60 min) with the HNO_3-$HClO_4$ mixture and the addition of HCl had no effect. However, although care was taken to minimize losses by using a long-necked (30 cm) flask, low recoveries were obtained for Pb, Se(IV), As(III), Cr(III) and Cr(VI) when HCl was used after $HClO_4$-HNO_3 digestion. Since Se(IV), As(III), Cr(III), Sn(II) and Sb(III) were oxidized by the acid mixture this fact has to be considered for some procedures and they have to be reduced before their determination. It was found that when HNO_3 was used alone or in an acid mixture for digestion, the recovery of Ni was only 40%. However, with the use of $HClO_4$ or H_2SO_4, the recovery was very high.

Key Words: Open wet digestion, losses, recovery, elimination, trace elements, differential pulse polarography.

Introduction

For the determination of trace element quantities in natural products, the material has to be digested first. Commonly used methods for digestion involve heating samples with mixtures of HNO_3, $HClO_4$, and H_2SO_4. However, HCl, HF, and H_2O_2 are also used, depending on the material and on the element under consideration. During the process of sample digestion by these methods, some elements were lost through volatilization,[1–4] even when a digestion bomb was used.[3] A newer technique uses microwave radiation for

*Corresponding author

sample digestion in high-pressure digestion vessels using HNO_3, HCl, H_2SO_4, and H_2O_2. Several laboratories use microwave radiation for sample dissolution process because it provides rapid sample dissolution. However, here too losses are unavoidable.[5−7]Trace elements in wine were determined by ICP-MS;[5] while some samples were digested in a microwave digestion system, some were not, for comparison. According to this work, low values of zinc in wine were indicative of partial loss during digestion with a microwave oven. In the same investigation, some digestion procedures including high-pressure ashing (HPA) and microwave (MW) digestion were compared with direct measurement without digestion for the recovery of trace elements. The recovery for nickel content was only 50% when MW and HPA were used. In a work for selenium speciation,[6] it was shown that above a certain power value in the microwave oven selenium was lost, presumably due to volatilization. Moreover, when long programs were used, low selenium recoveries were observed. During the microwave digestion of human urine in HNO_3 it was shown[7] that the digest contained incomplete digestion products.

For the trace element determination in mushrooms,[8] a closed microwave oven with concentrated HNO_3 has been used. Sodium persulfate, sodium fluoride, and nitric acid served as digestion reagents in a microwave digestion system[9] for total arsenic determination in biological samples. For the determination of Fe and Mn in pig liver, a microwave oven has been used with HNO_3 and H_2O_2.[10] A new sample treatment was proposed for wear metals in lubricating oil assisted by a microwave oven.[11] As can be seen, each material has to be digested according to its composition. Steel, for example, cannot be digested with HCl for its sulfur content since sulfur will be lost as H_2S. A sample for selenite determination has to be digested first with HNO_3 and $HClO_4$ but then HCl has to be used,[4,12] in order to reduce selenate to selenite. Thus, the digestion procedure will depend on the elements under consideration, on the method used for the determination, and on the volatility of the element.

The dependence of losses on digestion time has been studied for some trace elements in cauliflower. With 30 h of digestion all the selenium was lost. Similar losses were observed for Ti(IV), Cr(III) and Zn with longer (6-9 h) digestion times.[2] For the electrochemical determination of As and Se in tuna fish,[13] a wet digestion procedure of about 18 h using $HNO_3$and H_2SO_4 has been applied. After the addition of HCl and heating for 30 min more, the recovery was 84% for selenium.

Microwave digestion is excellent for routine analysis of known trace elements in well-known samples. However, there are many cases in which open wet digestion has to be used. With microwave ovens only 0.1-0.5 g sample quantities can be digested; thus the final volume and the concentrations will be small. When an unknown sample has to be analyzed for its trace element contents, wet digestion will be preferred since large amounts of sample such as 5-10 g can be digested at a time. Using larger quantities enables samples that reflect the average composition of the bulk of the material.

As can be seen from the literature, in all digestion procedures, either microwave oven or open wet digestion, there are cases of low recoveries. Thus, if the behavior of ions during digestion is known, precautions can be taken. The aim of this work was to investigate the effect of digestion time and type of acids on the recovery of elements. Wet open digestion was applied to 21 elements, using various acids and digestion times; care was taken to minimize losses. Differential pulse polarography (DPP) was used throughout the study for the trace element determinations.

Experimental

Apparatus

A polarographic analyzer (PAR 174 A) equipped with a PAR mercury drop timer was used. The drop time of the electrode was 2-3 s (2.35 mg s^{-1}). A Kalousek electrolytic cell with a saturated calomel electrode (SCE), separated by a liquid junction, was used in the three-electrode configuration. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY 1600) X-Y recorder under the conditions of a drop life of 1 s, scan rate of 2-5 mV s^{-1}, and pulse amplitude of 50 mV.

Reagents

All reagents used were of analytical reagent grade and all salts were chlorides except $MnSO_4$. Triply distilled water was used in the preparation of all solutions. Nitric acid (65%), perchloric acid (70%), and hydrochloric acid (37%) were used in the digestion procedure. The mercury (Analar) used in the dropping mercury electrode was obtained from BDH Chemicals Ltd., Poole, UK. Stock solutions (0.1 M) were used for the preparation of working solutions of 10^{-3} M by daily dilution. Contaminated mercury was cleaned by passing it successively through dilute HNO_3 (3.0 M) and water columns in the form of fine droplets using a fine platinum sieve. The collected mercury was dried between sheets of filter paper. A polarogram of this mercury was taken before use to ensure the absence of impurities. The same digestion period was applied to the same acid mixture without addition of trace elements and the polarogram was taken under the same conditions. No peak for impurity was observed.

Digestion procedure

For the digestion procedure, a long-necked (30 cm) 100 mL flask was used. Each time, a sample of ion under consideration was added to a solution of 10 mL of acid mixture ($HNO_3$and $HClO_4$ in 1:4 ratio) so that its concentration was 10^{-3} M. The acid was evaporated while stirring it on a Bunsen flame on an asbestos plate for 1 h and the acid left behind was about 1.0 mL. Then, according to the procedure, 1.0 mL of HCl was added and the evaporation time with HCl was taken for 5, 10, 20 and 40 min. Here the acids should not be vaporized until dryness because of larger losses. The same digestion procedure was applied for each ion without adding HCl and the recovery was checked once more. After the flask was cooled, distilled water was added until the volume was 10 mL. For some digestion methods only $HClO_4$ or H_2SO_4 had to be used in order to minimize losses. A sample of 0.1 mL was taken from the above-prepared 10 mL of solution and added to the polarographic cell; its concentration was 10^{-5}M. The DP polarogram was taken and with standard additions the quantity of ion present was determined.

Results and Discussion

Preliminary experiments

The effect of wet digestion on 21 ions, namely Zn, Cu, As(III), As(V), Se(IV), Se(VI), Cr(III), Cr(VI), Pb, Ni, Co, Mn, Sn(II), Sn(IV), V(III), Ti(IV), Fe, Cd, Sb(III), Sb(V), and Mo(VI), was studied. Digestion time, temperature and composition of acids were among the variables. The concentration of each ion was

determined with DPP after the digestion procedure. Since the supporting electrolyte was important for the determination, for each ion several electrolytes such as HCl, HAc/Ac^- and NH_3/NH_4Cl buffer were studied. The electrolyte in which the ion under consideration had the largest and best-defined peak was chosen as the working electrolyte for that ion. It was possible to determine 10^{-5}-10^{-6}M concentrations of the mentioned ions. While for many ions acetate buffer was suitable, for V(III), Co(II), Mn(II), and Ni(II) ions ammonia buffer, for V(III), Cr(III) and Cr(VI) ions K_2CO_3 solution, and for As(III), As(V), Sn(II) and Sn(IV) ions HCl solution was the most suitable electrolyte. The peak potentials and the best medium chosen are given in Table 1. As(III) and Sn(II) had larger peaks in HCl, and the peaks for Sn(II) and Sn(IV) could be separated better in this medium.

Table 1. Optimum conditions and E_p values for the DP polarographic determination of 21 ions.

Ions	Medium	pH	Ep (V)
Zn(II)	$HAc/NaAc^-$	2	-0.97
Cu(II)	$HAc/NaAc^-$	2	-0.14
Co(II)	NH_3/NH_4Cl	10	-0.24
Cd(II)	HAc/Ac^-	2	-0.64
Sb(III)	HAc/Ac^-	4 (EDTA)	-0.70
Sb(V)	No peak	-	No peak
Cr(III)	K_2CO_3	11.8	-0.87
Cr(VI)	K_2CO_3	11.8	-0.44, -0.87
Mo(VI)	HAc/Ac^-	2	-024
As(III)	0.1 M HCl	-	-0.43, -0.67, -0.82
As(V)	No peak	-	No peak
Ti(IV)	HAc/Ac^-	2 (EDTA)	-0.21
Ni(II)	HAc/Ac^-	4	-1.03
Mn(II)	NH_3/NH_4Cl	10	-0.65
Fe(III)	HAc/Ac^-	4	-0.05
V(III)	NH_3/NH_4Cl	10	-1.19
Se(IV)	HAc/Ac^-	2	-0.49
Se(VI)	No peak	-	No peak
Sn(II)	0.7 M HCl	-	-0.37, -0.70
Sn(IV)	0.7 M HCl	-	-0.75
Pb(II)	HAc/Ac^-	4	-0.40

Digestion losses

A solution containing Se(IV), As(III), and Zn each in 10^{-3}M concentration was digested with a (4:1) $HClO_4$:HNO_3 mixture. After evaporation of acids, HCl was added and it was evaporated in 40 min. The purpose of HCl addition was to reduce the Se(VI) formed during the digestion period since Se(VI) is not electro active. The polarogram taken in acetate buffer at pH 2 had only 2 peaks instead of 3 (Figure). The peak at about –0.48 V was for selenium, but its height was much lower than expected. The quantity of selenium determined by standard additions was about 60% lower than that present. The peak at about –1.0 V was for zinc and there was no loss during digestion. Arsenic, on the other hand, was not observed. These results indicate that care has to be taken, since each element can act differently during the digestion period. No arsenic peak was observed, since it was oxidized into As(V), which is not electro active. Although Se(IV) was also oxidized, it could be reduced with HCl and could be observed as a peak. As will be shown later,

the reason that the recovery for Se was very low (about 40%) was the HCl evaporation time. Thus, it can be concluded that for each ion these effects have to be considered.

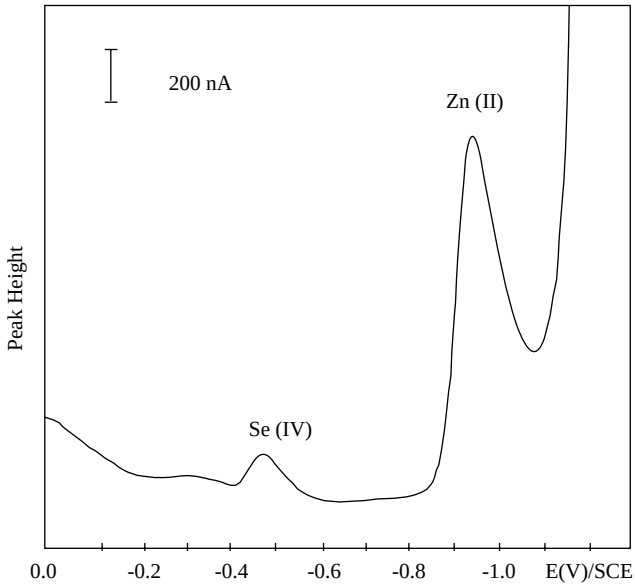

Figure. Polarogram taken after digestion of a mixture of 3 elements, 10^{-5}M As(III), 10^{-5}M Se(IV) and 10^{-5}M Zn(II), in acetate buffer pH 2.

To find the recoveries during digestion, each ion, in 1 x 10^{-3} M concentration, was added to 10.0 mL of acid mixture (4:1 HNO_3 and $HClO_4$) in the digestion flask. This flask had a long neck, about 30 cm, to minimize losses. The volume of the acid mixture was 10 mL, since this quantity is used generally to digest 1-2 g of biological material. First, for each ion, 60 and 100 min digestion times were applied, but, since there was no difference, 60 min was used throughout the experiments. Each digestion was carried out with and without HCl. HCl was added when about 1 mL from the acid mix was left after 60 min digestion. The time for the evaporation of HCl was 5, 10, 20, and 40 min for the ions that were affected during the evaporation. However, in most experiments, 10 min evaporation time was used. After digestion, the sample was diluted to 10.0 mL and from this solution 0.1 mL was taken and added to the polarographic cell containing the appropriate electrolyte (10 mL) solution for its measurement. Digestion was applied at least 3 times for each ion, and, for each digestion procedure, 3 polarographic measurements were performed. As can be seen from Table 2, some ions such as Zn, Cu, Cd, Ti(IV), Fe, Mo(VI), V(III), Co(II), and Mn(II) were not lost during digestion with or without HCl, and the recovery was about 100%. While the recovery was good for the above-mentioned ions, there were problems with some other ions; Cr(III) was oxidized to Cr(VI) but could be determined from this state. Some were oxidized to a state that was not electro active, such as Se(IV) to Se(VI), As(III) to As(V), and Sb(III) to Sb(V). Some of them were lost although care was taken to minimize losses during evaporation depending on the acid composition. The results for these ions are given in Table 3, which will be discussed separately.

Behavior of Se(IV) and Se(VI) ions

Previous studies[2,6] have shown that with longer digestion times the recovery for selenium was low, even with microwave digestion. In the present work, when Se(IV) was digested for 60 min and no HCl was used, no

peak was observed in the DPP polarogram, since it was oxidized to Se(VI), which is not electro active. In order to reduce it to Se(IV), HCl (1 and 2 mL) was added[12] after 60 min of digestion. Then it was evaporated in 5, 10, 20, and 40 min. When HCl was evaporated in 10 min, 100% recovery was obtained. However, the recovery was 50% and 85% after 40 and 20 min of evaporation, respectively. With long evaporation times, selenium will be lost as volatile selenium(IV) chloride.[4,13] When the evaporation of HCl was finished in 5 min the recovery of selenium(IV) was very small, about 50% was lost, indicating that the time for the reduction of Se(VI) was not sufficient. Thus, the reduction and evaporation of HCl should be around 10 min. The results are summarized in Table 3. Similar results were obtained for 1 and 2 mL additions of HCl, and thus 1 mL additions and 10 min of evaporation time were used throughout the experiments. A solution containing Se(VI) was also digested according to the same procedure and the recovery was 100% (the Se(VI) content was determined after reduction to Se(IV) with 1 mL of HCl for 10 min).

Table 2. Recoveries of ions after wet digestion with the HNO_3-$HClO_4$ mixture.

Element	Without HCl	With HCl				N*
	60 min /100 min	5 min	10 min	20 min	40 min	
Zn(II)	100 ± 2 /100 ± 3	97 ± 4	98 ±1	99 ± 2	99 ± 3	5
Cu(II)	100 ±1 / 99 ± 2	100 ± 1	98 ±1	98 ± 3	97 ± 3	4
Cd(II)	98 ± 3 /-		100 ± 4			3
Ti(IV)	99 ± 2 /-		97 ± 2			3
Fe(III)	99 ± 2 /-		100 ± 3			3
Mo(VI)	100 ± 2 /-		100 ± 3			3
V(III)	100 ± 2 /-		100 ± 3			3
Co(II)	100 ± 2 /-		100 ± 3			3
Mn(II)	98 ± 2 /-		100 ± 3			3

N*, number of measurements

Table 3. Recoveries of ions after wet digestion (HNO_3-$HClO_4$), with some problems.

Element	Without HCl	With HCl	N*			
	60 min /100 min	5 min	10 min	20 min 40 min		
Ni(II)**	41 ± 2 /-		40 ± 3			4
Cr(III)	75 ± 2 /-		40 ± 3			6
Cr(VI)	73 ± 2 /-		41 ± 3			4
Sn(II)	40 ± 2 /-		38 ± 3			4
Sn(IV)	39 ± 2 /-		41 ± 3			4
As(III)	100 ± 2 /-		98 ± 3			6
As(V)	100 ± 3 /-		99 ± 2			3
Se(IV)	No peak	46 ± 6	100 ± 4	84 ± 7	42 ± 5	6
Se(VI)	No peak		100 ± 4			3
Pb(II)	100 ±1 /100 ± 3	99 ± 1	84 ± 2	70 ± 1	32 ± 4	4
Sb(III)	99 ± 3 /-		100 ± 3			4
Sb(V)	98 ± 2 /-		97 ± 3			3

N*, number of measurements

**, recovery for Ni increased to 100% with $HClO_4$ and H_2SO_4

Behavior of nickel ion

The recovery was only 40% for nickel during digestion with and without HCl. It was concluded that these losses were because of volatile nitrate compounds of nickel. The same losses were observed for nickel in wine[5] with HNO_3. For this reason, instead of a HNO_3-$HClO_4$ acid mixture, different acids and acid combinations were studied. It was found that either 10 mL of H_2SO_4or 10 mL of $HClO_4$ alone or 10 mL of 1:1 H_2SO_4-$HClO_4$ mixture can be used. As can be seen from Table 3, in all of these acid conditions there was no loss of nickel ion. With H_2SO_4because of its slow evaporation the digestion took about 3 h. With an acid mixture of 1:1 H_2SO_4-$HClO_4$ it took about 1.5 h and with $HClO_4$ alone 1 h. Although $HClO_4$ evaporates more easily, for samples that cannot be digested with this acid, H_2SO_4 had to be added. It can be concluded that for nickel determinations in biological materials HNO_3 cannot be used for digestion.

During our differential pulse polarographic work for the determination of trace elements in reference hair,[14] when HNO_3 was used for its digestion the nickel content found was low.

However, when $H_2SO_4$was used during digestion the nickel could be determined without any loss.

The behavior of Cr (III) and Cr(VI) ions

In preliminary studies, the best electrolyte for Cr(III) was chosen as K_2CO_3, pH 12. In this electrolyte, Cr(III) has 1 peak at –0.87 V and Cr(VI) has 2 reduction peaks at –0.42 V and at –0.87 V; the first peak at –0.42 V was used for the determination of Cr(VI).

After digestion of Cr(III), instead of 1 peak at –0.87 V, 2 peaks at –0.42 and –0.87 V were observed, indicating the oxidation of Cr(III) to Cr(VI) during digestion. The losses of Cr(III) through volatilization can be calculated from the amount of Cr(VI) formed. Calculations showed losses of Cr(III), that is while the recovery was 75% after 60 min digestion (without HCl), it was 40% when HCl was used after digestion. The same digestion procedure was applied for Cr(VI) and the same losses were obtained. The recovery was 73% without HCl and 41% with HCl. Since Cr(III) was oxidized to Cr(VI) during digestion, the same evaporation behavior was expected. The results are given in Table 3 for both ions as deviations from the mean. It is seen that, when an acid mixture is used for digestion, losses are unavoidable.

Behavior of As(III) and As(V)ions

As(III) was oxidized during digestion to As(V) and since As(V) is not electro active it had to be reduced first with KI.[15] As(III) has 3 peaks in 0.1 M HCl electrolyte and the peak at –0.67 V was used because of its sharpness. No losses were observed for either ion after digestion. The recovery was about 100% with or without HCl. The results obtained are given in Table 3 as deviations from the mean.

Behavior of Sb(III) and Sb(V) ions

It was found that Sb(III) can be observed best in HAc- Ac buffer at pH 4 in the presence of EDTA, having a peak at –0.70 V. After digestion no peak was observed since Sb(III) was oxidized during digestion to Sb(V), which is not electro active. After reduction to Sb(III) with KI[15] it was determined and there was no loss for either case with and without HCl (Table 3).

Behavior of Sn (II) and Sn(IV) ions

During digestion Sn(II) is oxidized to Sn(IV) with nitric acid in the acid mixture. The Sn(IV) is electro active and can be observed with its reduction peak at –0.7 V in 0.7 M HCl. Thus there is no need to reduce Sn(IV), and the oxidized Sn(II) can be determined using this peak. The recovery was only about 40% after digestion for both Sn(II) and Sn(IV) ions with and without HCl.

Behavior of Pb(II) ion

There was no loss for lead ion when HCl was not used after 60 min evaporation of the acid mixture, but when HCl was used the losses were large. The recovery varied with the evaporation time of HCl: it was 32%, 70%, 84%, and 99% for 40, 20, 10, and 5 min evaporation times, respectively (Table 3). These results show consistency with the volatility of lead chlorides. Thus, for lead ion determination it is better not to use HCl; however, when it has to be used the evaporation of HCl should be about 5 min. Care has to be taken when H_2SO_4 is used for digestion. Because of $PbSO_4$ precipitation low recoveries[3] will be obtained. In this case the filtered precipitate has to be washed with hot water.

Conclusions

For the determination of trace elements in biological materials, these samples have to be digested first. Wet digestion or microwave ovens are used for this purpose. In both cases, care has to be taken in order to avoid losses. Since in many digestion procedures acids are used, their effect on digestion has to be known. In this work, the importance of the composition of the acid mixture and digestion time was investigated. When HCl was used during digestion, chromium and lead ions were lost in significant amounts. In this case, either HCl should not be used during digestion or if it has to be used the evaporation time of HCl must be as short as possible.

For the determination of Ni ion, on the other hand, HNO_3 cannot be used because of the volatility of its nitrates. $HClO_4$and H_2SO_4 are suitable acids for the digestion of biological materials containing Ni. They can be used either alone or as a mixture. This fact is shown in the determination of Ni in reference hair.

It has to be taken into account that Se, As, Sb, Sn, and Cr are oxidized and thus either a suitable method for their determination has to be applied or they have to be reduced first. If HCl is used for the reduction of Se(VI), the evaporation time of HCl has to be about 10 min. With longer evaporation times it will be lost as volatile Se(IV) chloride, but shorter times will not be sufficient for the reduction of Se(VI).

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