Determination of Fe, Cu, Ni, and Mn in Fats and Oils by Flameless Atomic Absorption Spectroscopy

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ABSTRACT

The determination of Fe, Cu, Ni, and Mn is performed by flameless atomic absorption spectroscopy. The procedure is applicable to oils, including crudes, and also fats such as hydrogenated oils. A solution of the sample is analyzed directly, and absorbance values are compared to standards. Matrix-matching is not required, and virtually any concentration of metal can be analyzed since both sample concentration and aliquot volume are variable. Solvent selection is based on the results of a stability study and includes methyl isobutyl ketone (MIBK) for Cu and Mn determinations and MIBK with a small amount of HNO_3 added for Fe and Ni. Sensitivity approximated 80 picograms for Fe, 100 pg for Cu, 525 pg for Ni, and 30 pg for Mn. Absorbance values of appropriate aliquots, each having the equivalent of 0.10 ppm Fe, are compared to determine the effect of variation of pipette size on response. Pipette size ranged from 5 μl to 50 μl. Responses using multiple 50 μl volumes are also recorded. Sources of error in procedure, error analyses, and proper pipetting technique are given.

INTRODUCTION

Because of the possible detrimental effects of many heavy metals on the stability of fats, oils, and related products, it has become necessary to develop reliable methods for the development of an atomic absorption method for the analysis of Fe, Cu, and Ni, and Mn in liquid oils and high melting fats such as hydrogenated oils (1).

Although atomic absorption spectroscopy (AAS) has been used extensively in the industry for the analysis of metals in fats and oils, established procedures utilize flame atomic spectroscopy (2,3).

Flame spectroscopy, utilizing techniques such as charashing and wet ashing for aqueous matrices and solvent extraction and chelating agents for organic matrices, is useful in many applications but has certain limitations (relatively poor sensitivity, time-consuming sample preparations, large sample size, interferences) (4-8). Black (4) compared dry ashing, solvent dilution with direct aspiration, and carbon rod flameless determinations and discussed advantages and disadvantages of each. The carbon rod and graphite furnace are competitor's products, and, while no attempt to directly compare them was made, certain limitations of the carbon rod atomizer encountered by Black were not present with the furnace, namely, incomplete atomization for certain elements and limited sample volume (1 μl).

EXPERIMENTAL PROCEDURES

Apparatus

A Perkin-Elmer 306 Atomic Absorption Spectrophotometer equipped with an HGA-2000 graphite furnace, deuterium arc background corrector, optical modification kit, and a Perkin-Elmer 56 strip chart recorder was used.

Furnace operating conditions used in all determinations included

Cycle	Time (sec)	Temperature (C)
Dry	30	100
Char	20	800
Atomization	20	2300

Conditions for the drying step are suitable for the removal of the solvent matrix, and atomization conditions are based on the near optimum temperature (maximum absorbance). Although the four metals investigated had slightly different optimum atomization temperatures, one temperature (2,300 C) was chosen for convenience.

In establishing the char conditions, it was necessary to consider the following:

1. A temperature of 400 C is required to remove the organic (lipid) material in a reasonable period of time.
2. 1,200 C is generally regarded as the maximum char temperature for the four elements of interest (see Perkin-Elmer Graphite Furnace Manual).

Smoke was present at atomization even when the char temperature was increased to 1,200 C for periods up to 2 min. This indicated that atomization temperatures were required for complete removal of the last traces of organic material.

The HGA-2000 accessory has the capability of automatically interrupting the flow of purge gas at atomization to increase sensitivity by lengthening the time atoms of interest are in the tube. In this work, argon was used as the purge gas without flow interruption.

The deuterium arc background corrector was used in all determinations. Perkin-Elmer "Intensitron" hollow cathode lamps were used with the following instrumental parameters:

Elements	Wavelength (nm)	Slit (nm)
Fe	248.3 UV	0.2
Cu	324.7 UV	0.7
Ni	232.0 UV	0.2
Mn	279.5 UV	0.2

"Grooved" graphite tubes were used rather than the standard "straight" tubes. Tube lifetime averaged 150 determinations.

Reagents

The following Eastman organometallics were used to prepare the organic standard solutions:

1. Manganous cyclohexanebutyrate
2. Cyclohexanebutyric acid copper salt
3. Cyclohexanebutyric acid nickel salt
4. Tris (1-phenyl-1, 3-butanediono) iron III

Xylene, methyl isobutyl ketone (MIBK), and nitric acid used in solutions were reagent grade.

Procedure

Preliminary work involved the preparation of 500 ppm organic solutions of Fe, Cu, Ni, and Mn in xylene and ethylhexane acid according to Eastman directions. From these stock solutions, 100 ppm solutions were prepared. Both were stored in Pyrex glass containers.

The 100 ppm standards were convenient for the prepa-

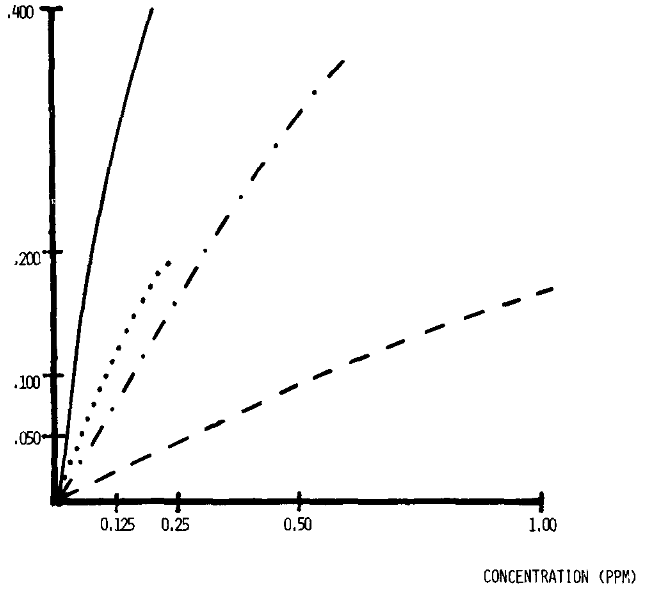

FIG. 1. Absorbance vs. metal concentration. (◦•◦◦) Fe in MIBK + HNO_3; (-•-◦) Cu in MIBK; (----) Ni in MIBK + HNO_3; (——) Mn in MIBK.

rations of the low concentration working standards required in typical analyses.

To prepare, for example, a 0.1 ppm standard, 10 µl was dispensed with an Eppendorf pipette into a stabilized 10 ml volumetric flask and made to volume. Flasks were stabilized by storing working standards prior to use. To prepare other concentrations, different pipette volumes were used, maintaining the 10 ml flask volume. New tips with no prior wetting were used for each preparation.

The following procedure was used in the analysis of an oil:

A 1 g sample was weighed into an acid washed 10 ml volumetric flask. Depending on the element of interest, the most suitable solvent system was chosen to ensure stability for the duration of the analysis. The solvent systems selected were MIBK for Cu and Mn, and MIBK + five drops concentrated HNO_3/10 ml solution for Fe and Ni.

A fresh standard was prepared using the same solvent system.

Twenty microliter aliquots of the standard were injected into the furnace, and the average of three determinations which agree to ± 0.005 absorbance units was recorded. Typically, the concentration of the standard was 0.1 ppm, since this approached the upper limit of linearity for Fe. This procedure was repeated for a 10 µl aliquot, the equivalent of 0.05 ppm. If feasible, a third aliquot was determined. A plot of absorbance vs. concentration established the linearity (Fig. 1).

Next, 20 µl of the sample were injected, and the average of three determinations agreeing to ± 0.005 absorbance units was recorded. If the signal produced was beyond the linear portion of the curves presented in the figures, either a smaller aliquot was taken or a smaller sample size was used. Interpolation from the standard curve produces the concentration of the element in the oil.

Another method of calculation used was the method of additions. Three identical solutions of the sample were prepared. To one, 0.05 ppm of the element of interest was added with an Eppendorf pipette (5 µl of 100 ppm stock solution). To a second, 10 µl or 0.1 ppm was added. The three solutions were analyzed, and the averages of the determinations recorded. It should be noted that the concentrations of the solutions must be low enough to produce a linear signal in all determinations. A plot of concentration vs. absorbance produces a line which intersects the negative x axis (concentration) at x_b (Fig. 2). The difference between x_b and x_a (the point of x-axis intercept of the standard curve) yields the value, which, when diluted factors are applied, is the concentration of the metal in the oil. The "method of additions" approach is valuable since any influence of sample matrix on response is essentially eliminated.

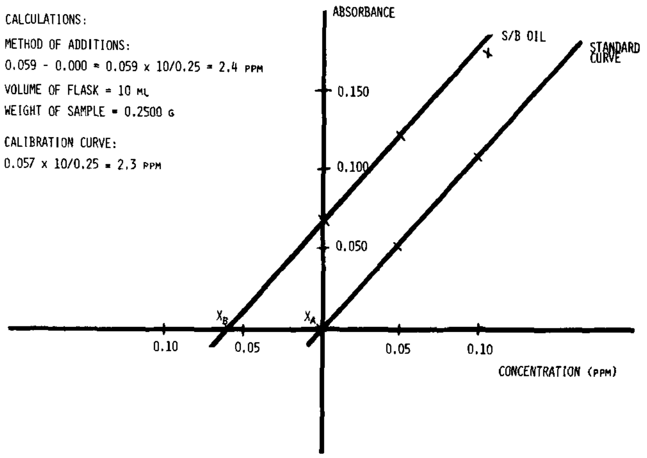

FIG. 2. Absorbance vs. metal concentration. Comparison of calculations.

RESULTS AND DISCUSSION

While attempting to develop a method for the determination of metals in oils using the graphite furnace, it was learned that choice of graphite tube type was an important consideration. When standard straight tubes were employed, the low surface tension organic liquids flowed the length of the tube, sometimes reaching the cone connections even at 20 µl volumes. The "grooved" tubes, however, were very satisfactory since volumes up to 50 µl are contained in the groove at the middle of the tube. The two major disadvantages of the grooved tube (lower sensitivity and lower maximum temperature) were tolerated.

Another factor contributing to a lack of reproducibility, especially at low concentrations, was background noise due to emission by the glowing walls of the graphite tube at atomization. The incorporation of the Perkin-Elmer optical modification kit accessory eliminated this problem, greatly improving detection limits (twice the mean of the blank readings) (Table I).

Although the above modifications allowed for generally improved and acceptable results in the analyses of standards, determinations of oil solutions were unsatisfactory. Typically, consecutive determinations of identical aliquots of a sample solution containing 10% oil in xylene yielded absorbance values ranging from detection limit amounts to readings exceeding 1.000 absorbance.

It was also observed that a considerable amount of smoke was present at atomization. Even with background correction in operation, smoke was an area of concern. Extending the char cycle to periods up to 6 min and temperatures to as high as 1,200 C reduced the amount of smoke present at atomization; however, no improvement in signal reproducibility was realized. A measure of the "smoke peak" using a nonabsorbing Fe line indicated that the amount of smoke present at atomization using the original char conditions was within acceptable limits (ca. 0.700 absorbance). An alignment check of the D_2 arc and variations in element lamp alignment was performed to improve focal point alignment. This had little effect.

The gas interrupt option on the HGA-2000 has been described above. Its employment in the determination of organic Fe standard increased the sensitivity by 100%.

TABLE I

Detection Limits of Graphite Furnace

	With optical modifications (ng)	Without optical modifications (ng)
Fe	0.1	0.25
Cu	0.1	0.25
Ni	0.25	2.0
Mn	0.05	0.15

Increases were also realized for several other metals tested.

When gas flow was not interrupted during atomization, by placing the option in the manual position, reproducible results were obtained for all four metals in oil-containing matrices, though with some loss in sensitivity.

Interruption of the gas allows strata of smoke to remain in the tube at atomization. Although the amount of smoke present is within correctable limits as mentioned previously, the heterogeneous and irreproducible nature of the strata make successful background correction difficult to achieve. On the other hand, gas flow tends to homogenize the smoke in addition to accelerating its exit from the tube and, therefore, is a very important consideration.

To determine the stability and the most suitable solvent system for standard solutions and also the solutions of the oil for analysis, a study followed in which standard solutions of Fe, Cu, and Ni at a 0.1 ppm level and Mn at a 0.05 ppm level were prepared in three solvents: xylene, MIBK, and MIBK + five drops of HNO_3 per 10 ml volume.

In addition, solutions of an oil were prepared in each of the three solvent systems. Crude palm oil was used in the Cu and Mn tests, treated palm oil in the Fe tests, and hydrogenated soybean oil in the Ni tests.

Samples were checked at zero time and at 1, 2, 6, 24, and 48 hr intervals. The values presented in Table II represent the absorbance for 20 μl aliquots.

Based on the stability data, the following systems were chosen for the respective metals:

1. Fe–MIBK + HNO_3

   Xylene is also acceptable; however, solutions in MIBK are apparently somewhat unstable and absorbance readings are slightly depressed. While variability between zero time values of an element is due in part to pipetting error (Table III), some is possibly solvent-related.

2. Cu–MIBK

   While xylene is unacceptable as the solvent for Cu determinations, either MIBK or MIBK + HNO_3 have good stability–the former being the choice in work presented in this report.

3. Ni–MIBK + HNO_3

   Neither xylene nor MIBK is acceptable due to instability.

4. Mn–MIBK

   While it is slightly unstable, MIBK is the best selection of the three systems.

TABLE II

Stability of Absorbance Readings in Standard and Oil Solutions

	Absorbance (hr)					
	Zero	1	2	6	24	48
Fe standard						
0.1 ppm Fe in xylene	0.110	0.106	0.106	0.106	0.104	0.102
0.1 ppm Fe in MIBK[a]	0.086	-[b]	0.088	0.088	0.076	0.078
0.1 ppm Fe in MIBK + HNO_3	0.099	-	0.095	0.095	0.096	0.096
Fe in						
oil in xylene	0.114	-	0.096	0.108	0.106	0.105
oil in MIBK	0.092	-	0.086	0.086	0.080	0.096
oil in MIBK + HNO_3	0.096	-	0.092	0.094	0.093	0.090
Cu standard						
0.1 ppm Cu in xylene	0.120	0.092	0.078	-	0.044	-
0.1 ppm Cu in MIBK	0.102	0.098	0.098	0.098	0.094	0.090
0.1 ppm Cu in MIBK + HNO_3	0.112	0.110	0.116	0.114	0.114	0.116
Cu in						
oil in xylene	0.012	0.012	0.012	-	0.012	0.012
oil in MIBK	0.010	0.010	0.010	-	0.010	0.010
oil in MIBK + HNO_3	0.010	0.010	0.010	--	0.010	0.010
Ni standard						
0.1 ppm Ni in xylene	0.017	-	0.009	0.004	-	-
0.1 ppm Ni in MIBK	0.024	-	0.018	0.016	0.013	0.011
0.1 ppm Ni in MIBK + HNO_3	0.023	-	0.022	0.023	0.025	0.024
Ni in						
oil in xylene	-	-	-	-	-	-
oil in MIBK	0.056	-	0.055	0.050	0.055	0.054
oil in MIBK + HNO_3	0.050	-	0.050	0.052	0.053	0.051
Mn standard						
0.05 ppm Mn in xylene	0.148	-	0.112	0.062	0.012	0.004
0.05 ppm Mn in MIBK	0.164	-	0.158	0.150	0.146	0.150
0.05 ppm Mn in MIBK + HNO_3	0.128	-	0.110	0.088	0.078	0.064
Mn in						
oil in xylene	0.084	-	0.078	0.070	0.078	0.074
oil in MIBK	0.088	-	0.082	0.082	0.088	0.084
oil in MIBK + HNO_3	0.070	-	0.068	0.062	0.062	0.060

[a] MIBK = methyl isobutyl ketone.

[b] (-) indicates no reading was taken.

In an actual analysis, standards should be checked occasionally and remade if necessary.

While investigating the need for matrix-matching standards and oil samples, the method of additions curves for various oils indicated that no matching is necessary. The responses (absorbances) of standards and standards added to the oils were essentially equal (Fig. 2). This was true in all cases, regardless of oil type.

Sensitivity for the HGA is defined as the mass of the element required to give a peak absorbance of 0.0044. Using this definition, values approximated 80 pg for Fe, 110 pg for Cu, 525 pg for Ni, and 30 pg for Mn.

In the analysis of low-melting triglycerides, a 10% (w/v) solution of the sample is suitable since the resulting viscosity, surface tension, and smoke at atomization are within acceptable limits.

It is also necessary to determine the metals content of substances with high melting points, such as stearines and emulsifiers. Due to solubility problems, the 10% solutions may be unattainable and solutions as low as 2% may be necessary.

To obtain higher absorbance values, larger aliquots of the sample solution are necessary. An experiment to validate the use of large and also multiple aliquots was designed.

The following concentrations of organometallic Fe were made in MIBK + HNO_3 (five drops of concentrated HNO_3 per 10 ml MIBK): 0.01, 0.02, 0.05, 0.10, 0.20, and 0.40 pg/μl; 200, 100, 40, 20, 10, and 5 μl aliquots were taken, respectively. Volumes over 50 μl represent multiple aliquots. For example, to achieve 200 μl, four 50 μl aliquots were used with the solvent removed after each injection by the drying stage of the furnace. Absorbance values for three sets of experiments are listed in Table IV.

TABLE III

Pipette Accuracy Based on Reproducibility of Absorbance Values

5 μl Pipette–0.05 ppm Fe in MIBK[a] + HNO_3

Standard number	Mean of four absorbance values	Standard deviation[b]
1	.062	.003
2	.063	.003
3	.058	.003
4	.058	.003

10 μl Pipette–0.10 ppm Fe in MIBK + HNO_3

Standard number	Mean of four absorbance values	Standard deviation[c]
1	.107	.003
2	.109	.003
3	.101	.003
4	.103	.003

[a] MIBK = methyl isobutyl ketone.

[b] Standard deviation between groups 0.003; average standard deviation within groups 0.005.

[c] Standard deviation between groups 0.003; average standard deviation within groups 0.006.

Statistical analysis of the absorbance values indicated that a noticeable difference exists between values obtained using different pipette aliquots. A careful consideration of the technique involved in using the micropipette offers an explanation for the differences encountered.

Due to capillary action, a noticeable volume of low surface tension liquids is drawn up into the disposable tip of the pipette. A measure of the absorbance when this volume was injected into the instrument indicated that it is 0.6 μl for MIBK. Because the volume is independent of pipette size, the resulting error increases with decreasing

TABLE IV

Absorbance Values of 0.1 ppm Fe Solutions Using Combinations

Experiment	Volume (μl)	Concentration (ng/μl)	Mean of four absorbance values	Standard deviation
1	40	.05	.120	.003
	20	.10	.116	.002
	10	.20	.112	.001
	5	.40	.134	.002
2	100 (50 x 2)	.02	.122	.002
	40	.05	.133	.002
	20	.10	.125	.002
	10	.20	.122	.002
	5	.40	.132	.003
3	200 (50 x 4)	.01	.126	.004
	100 (50 x 2)	.02	.121	.002
	40	.05	.133	.003
	20	.10	.120	.001
	10	.20	.119	.004
	5	.40	.132	.002

TABLE V

Error Resulting from Improper Pipetting Technique

Standard preparation						
Desired concentration of solution (ppm)	.010	.020	.050	.100	.200	.400
Pipette required per volumetric size (μl/ml)	5/50	10/50	5/10	10/10	20/10	40/10
Actual concentration due to 0.62 μl error	.011	.021	.056	.106	.206	.406
Percent of desired concentration	110	105	112	106	103	102
Injection of aliquot						
Pipette used to inject aliquot (μl)	50 x 4	50 x 2	40	20	10	5
Actual volume of injection (μl)	202.5	101.2	40.6	20.6	10.6	5.6
Percent of desired volume injected	101.3	101.2	101.5	103	106	112
Actual concentration of injected solution	.011	.021	.057	.109	.218	.455
Percent error	10.0	5.0	14.0	9.0	9.0	13.8

TABLE VI

Absorbance Readings Using "Improved" Pipetting Techniques

Experiment	Volume	Concentration (ng/μl)	Mean of four absorbance values	Standard deviation
1	200	.01	.118	.002
	100	.02	.121	.002
	40	.05	.121	.003
	20	.10	.120	.003
	10	.20	.118	.002
	5	.40	.118	.002
2	200	.01	.119	.002
	100	.02	.121	.003
	40	.05	.119	.003
	20	.10	.120	.002
	10	.20	.117	.002
	5	.40	.118	.002
3	200	.01	.118	.002
	100	.02	.121	.003
	40	.05	.118	.002
	20	.10	.117	.002
	10	.20	.121	.002
	5	.40	.121	.002

pipette size. Since different size pipettes are used both in making up the standard solutions and for aliquot injections, the error encountered is complex. The complete analysis is presented in Table V.

To eliminate the above source of error, the following technique is used:

1. Depress the knob on the pipette to the first stop.
2. Immerse the tip into the liquid and slowly release the knob.
3. To empty the tip, press the knob down to the first stop, maintaining contact between the end of the tip and the inside of the vessel.
4. Remove the tip carefully from the vessel. The residual volume approximates the volume drawn up by capillary action.

Repeat of the experiments using the above technique indicated variation of pipette size has no significant effect on absorbance. Results are presented in Table VI. Results in Table VI also demonstrate that no significant differences are observed when using large multiple aliquots as opposed to the standard single injection volume of 5-20 μl sample volume.

To determine the error in preparation of a standard from a stock solution, the following experiments were performed. Four 0.05 ppm Fe standards were prepared in MIBK + HNO_3 by pipetting 5 μl of 100 ppm Fe stock solution into 10 ml volumetric flasks. Twenty microliter aliquots of the standard were injected into the furnace, and the average of four determinations was recorded.

The absorbance values of the four standards were checked for agreement as presented in Table III. The standard deviation between groups was 0.003, and the average standard deviation within groups was 0.005.

Four 0.1 ppm Fe standards were prepared using the above procedure and a 10 μl pipette. The absorbance values of these four standards gave a standard deviation between groups of 0.003, and the average standard deviation within groups was 0.006.

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