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Preparation and Analysis of Standardized
Waste Samples for Controlled Ecological
Life Support Systems (CELSS)

John L. Carden
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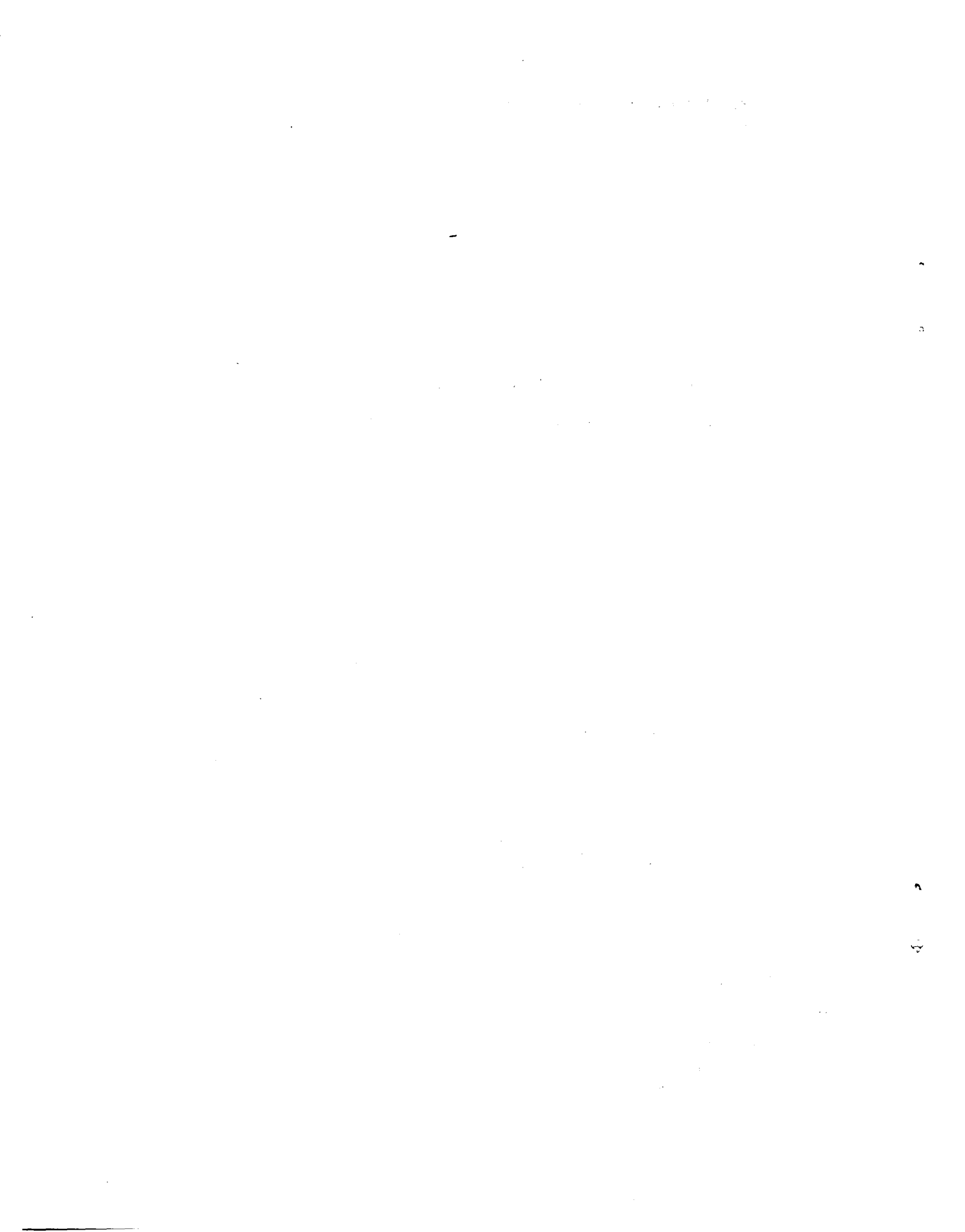
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CELSS-RELATED
CHEMICAL MEASUREMENTS RESEARCH AT GEORGIA TECH:

A Summary of activities between January 1, 1981, and March 1, 1982.

1.0 Introduction

During the past eighteen months our efforts have focused on four areas:

1. Preparation and analysis of standard materials for use by CELSS researchers,
2. Analysis of samples from Professor Modell's wet oxidation experiments,
3. Development of ion chromatographic techniques utilizing conventional HPLC equipment, and
4. The investigation of techniques for interfacing an IC (ion chromatograph) with an ICPOES (inductively coupled plasma optical emission spectrometer).

We also performed limited elemental analyses on a series of samples from Dr. Bruce Onisco's (formerly of NASA Ames Research Center) batch wet-oxidation experiments.

During this period Professor Richard Browner of the School of Chemistry at Georgia Tech became involved in CELSS-related research. Professor Browner is an analytical chemist with an international reputation in atomic spectroscopy.

Our work involving elemental analysis has been seriously hampered by problems with our ICPOES system. The original instrument (model ICP 5000 manufactured by the Perkin-Elmer Corporation) was an early production integrated sequential ICPOES and AAS (atomic absorption spectrometer)

system. The advantages of this integrated approach are substantial including the ability to analyze for trace quantities of all but four or five of the known elements and the availability of both emission and absorption spectrometric modes in the same instrument. Unfortunately, a series of hardware problems has prevented us from fully investigating and capitalizing on these capabilities. Shortly after the instrument was delivered, a problem developed in the RF power supply for the plasma torch. Ultimately, the power supply and torch assembly had to be returned to Norwalk and replaced with new units. The monochromator assembly developed a problem in the wavelength drive subsystem which could not be repaired in the field, and Perkin-Elmer elected to replace the monochromator with a new unit incorporating improvements in the wavelength drive assembly. Significant problems were also experienced with the computer based instrument control and data system and with the graphite furnace power supply. The replacement monochromator system exhibited intermittent noise in the analyte signal and calibration drift, consequently. Perkin-Elmer has elected to replace the entire system with a model ICP 5500. The problems we have experienced with this new type of instrumentation have been a source of great frustration, slowing our research and making the acquisition of high-quality analytical data very tedious. Despite these frustrations we have accomplished our major goals, and we hope to soon have a fully functional ICPOES.

2.0 Preparation and Analysis of Standard Materials for CELSS Researchers

Standard materials prepared and/or analyzed during this period included:

1. freeze-dried human feces
2. freeze-dried human urine

- 3 freeze-dried inedible food preparation waste; Batch 1
4. freeze-dried inedible food preparation waste; Batch 2
5. Serafil (young rye)

Small batches of freeze-dried feces and urine were prepared and distributed prior to this period. Information about these materials can be found in our last progress report on NASA grant NSG 2403. This report deals with the preparation of the final batches of these materials.

The standard materials were analyzed for B, C, Ca, Cu, Fe, H, K, Mg, Mn, N, Na, S, Si, V, and Zn. The analyses for B, Ca, Cu, Fe, K, Mg, Mn, Na, Si, V and Zn were carried out by ICPOES while C, H, and N were determined using a Perkin-Elmer Model 240 CHN Analyzer, and S was determined using a modified Dumas method.

Analysis by ICPOES requires that the sample be in solution and free of suspended solids. An extensive literature search was carried out to find an adequate method to digest the solid standard materials. The most promising wet ashing techniques were tried, including the nitric acid-hydrogen peroxide technique outlined in our last progress report on NSG 2403. All of these techniques posed two serious problems for our applications: the feces, Serafil, and food preparation wastes either did not digest completely or formed a fine white precipitate upon dilution to final volume and all digestates were strongly acidic, requiring at minimum a ten to one dilution to bring the concentration of acid into a range acceptable for use in our instrumentation.

In hopes of overcoming these problems we evaluated a simple dry ashing technique consisting of heating from 100 mg to 1 g of sample contained in a covered porcelain crucible to 500°C in a muffle furnace and holding at this temperature for four hours. We hoped this technique would produce complete

ashing without significant analyte loss. Table 1 shows the results of our analyses of NBS "orchard" and "spinach" leaves ashed using this technique. With the exceptions of K and Cu the results agree to within the uncertainties in the NBS certified values. We strongly suspect that the problem with the K results is instrumental and not a result of the ashing process. Others¹ using this technique have reported copper contamination from porcelain crucibles, and this conclusion appears to be supported by our results.

The dry ashing technique produced particle free solutions (the residue was taken up in 5% HNO₃) for all of the standard materials except Serafil. Serafil left a fine white residue which did not dissolve. We suspect this solid is SiO₂.

Since this technique provided acceptable recoveries for most of the elements of interest and was successful in ashing the matrices involved, it was incorporated into the sample preparation protocol of the analyses which follow.

2.1 Freeze-Dried Human Feces

Working under a cooperative agreement, the Human Nutritional Research Laboratory of the U. S. Department of Agriculture, located at Grand Forks, North Dakota, collected and freeze-dried feces and urine samples for us. These samples were collected during transitional periods when the diets of their volunteer subjects were being modified.

¹ Zeitlin, H. et al., Anal. Chem., 30, 1284 (1958)

Table 1: Results of analyses of NBS standard reference materials following dry ashing.

NBS STANDARD REFERENCE MATERIAL	ELEMENT	OBSERVED CONCENTRATION $\mu\text{g/g}$	CERTIFIED CONCENTRATION $\mu\text{g/g}$	PERCENT ERROR	WITHIN LIMITS
SRM 1571	B	31 \pm 7	33 \pm 3	-6	yes
"Orchard Leaves"	Ca	19,000 \pm 2,000	20,900 \pm 300	-9	yes
	Cu	26 \pm 9	12 \pm 1	+120	no
	Fe	270 \pm 30	260 \pm 10	4	yes
	K	11,000 \pm 2,000	14,700 \pm 300	-25	no
	Mg	5,500 \pm 500	6,200 \pm 200	-11	yes
	Mn	89 \pm 11	91 \pm 4	-2	yes
	Zn	25 \pm 4	25 \pm 3	0	yes
SRM 1570	B	27 \pm 4	30*	-10	yes
"Spinach Leaves"	Ca	14,000 \pm 1,000	13,500 \pm 300	+4	yes
	Cu	33 \pm 3	12 \pm 2	+175	no
	Fe	520 \pm 50	550 \pm 20	-5	yes
	K	32,000 \pm 3,000	35,600 \pm 300	-10	no
	Mn	180 \pm 20	165 \pm 6	+9	yes
	Zn	51 \pm 5	50 \pm 2	+2	yes

* not certified by NBS

The particle size distribution of the feces as received was such that a very large aliquot was required to obtain a representative sample. Since some CELSS researchers utilize samples on the order of a few hundreds of milligrams, it was necessary to significantly reduce particle sizes and to insure adequate mixing of the entire batch. This was achieved by grinding 100-g lots of the feces in a Waring commercial blender, sieving through a 20-mesh grid, combining all lots into a large polyethylene bag and "shaking" to produce uniform mixing. The homogenized batch was divided into 100-g samples and packaged in tight-sealing, wide-mouth polyethylene bottles. All of these operations were carried out in an externally supported glove bag setup in a properly functioning laboratory fume hood. The glove bag was supplied with dry compressed air to minimize moisture absorption. It was necessary to locate the glove bag in a fume hood to prevent escape of fecal particulate into the laboratory when the glove bag seal was broken. As an added precaution personnel working in the hood always wore a surgical-type dust mask. A 0.5% NaOCl solution was used to disinfect all surfaces, containers, etc. contaminated with feces.

A total of four samples were taken from the master batch, dry ashed, taken up into 5% HNO₃, and analyzed by ICPOES. The results of these analyses as well as those for C, H and N are given in Table 2. Chloride ion content was determined using a Corning ion specific electrode. The chloride value reported in Table 2 was obtained using the method of standard additions to reduce matrix effects.

2.2 Freeze-Dried Human Urine

The USDA Human Nutritional Research Laboratory collected and freeze-dried the urine excreted by the experimental group during the period that our fecal material was collected. The urine as received also required

Table 2: Elemental composition of the CELSS standard material "feces."

<u>ELEMENT</u>	<u>AVERAGE COMPOSITION ($\mu\text{g/g}$)</u>
Mg	6,600 \pm 600
Ca	25,000 \pm 3,000
Na	18,000 \pm 1,000
K	28,000 \pm 4,000
Zn	270 \pm 40
Cu	40 estimate
Mn	170 \pm 14
V	6 estimate
Si	400 \pm 40
B	15 \pm 1
Fe	430 estimate
C	41.92%
H	6.59%
N	8.26%
Cl ⁻	2.1%

grinding for particle size reduction and careful mixing. All operations on the urine sample had to be carried out in the dry atmosphere of the glove bag because of the hygroscopic nature of the material. Three samples were taken from the master batch and prepared for analysis following the protocol used for the feces. The results are given in Table 3. Chloride ion content was determined using a Corning ion specific electrode. The chloride value report in Table 3 was obtained using the method of standard additions to reduce matrix effects.

2.3 Inedible Food Preparation Waste

Professor Marcus Karel of Food Sciences at MIT prepared two batches of a model inedible food preparation waste. Our last progress report on NSG 2403 contains a section contributed by Professor Karel characterizing this material. Grinding and mixing was not necessary. Three samples were taken from each batch and analyzed. The results are given in Tables 4 and 5.

2.4 Serafil

Professor Michael Shuler of Chemical Engineering at Cornell obtained a large batch of Serafil, a commercial material prepared by milling young rye plants. The material was adequately ground as received. The individual lots were combined into a master batch and repacked into 100-g containers. Six samples of Serafil were taken for analysis, and the results are given in Table 6.

3.0 Analysis of Samples from MIT Wet-Oxidation Experiments

Table 7 contains our results from the analysis of samples from MIT. A discussion of these results will be included in the next report on NSG 2403.

Table 3: Elemental composition of the CELSS standard material "urine."

<u>ELEMENT</u>	<u>AVERAGE CONCENTRATION ($\mu\text{g/g}$)</u>
Mg	2,100 \pm 200
Ca	4,500 \pm 200
Na	68,000 \pm 6,000
K	42,000 \pm 5,000
Zn	13 \pm 4
Cu	19 \pm 4
Mn	ND
Fe	7 \pm 3
V	4 \pm 2
Si	150 estimate
B	14 \pm 3
C	17.58%
H	4.93%
N	21.69%
S	1.80%
Cl ⁻	1.6%

Table 4: Elemental composition of CELSS standard reference material "Food Preparation Waste #1."

<u>ELEMENT</u>	<u>AVERAGE CONCENTRATION ($\mu\text{g/g}$)</u>
Mg	4,600 \pm 200
Ca	19,000 \pm 1,000
Na	710 estimate
K	7,400 \pm 600
Zn	78 \pm 20
Cu	31 estimate
Mn	170 \pm 20
Fe	150 \pm 15
V	6 estimate
Si	1,100 \pm 300
B	13 \pm 2
C	43.93%
H	6.34%
N	2.40%

Table 5: Elemental composition of CELSS standard reference material "Food Preparation Waste #2."

<u>ELEMENT</u>	<u>AVERAGE CONCENTRATION ($\mu\text{g/g}$)</u>
Mg	4,800 \pm 300
Ca	15,000 \pm 600
Na	1,000 estimate
K	8,600 \pm 1,000
Zn	77 \pm 14
Cu	26 estimate
Mn	160 \pm 16
Fe	130 estimate
V	5 estimate
Si	660 estimate
B	10 \pm 2
C	43.55%
H	6.45%
N	2.87%

Table 6: Elemental composition of CELSS standard material "Serafil."

<u>ELEMENT</u>	<u>AVERAGE COMPOSITION ($\mu\text{g/g}$)</u>
Mg	1,100 \pm 200
Ca	3,900 \pm 500
Na	1,300 \pm 400
K	41,000 \pm 4,000
Zn	29 \pm 6
Cu	13 \pm 4
Mn	84 \pm 9
V	3 estimate
Fe	460 estimate
Si	did not digest
B	8 estimate
C	40.63%
H	5.57%
N	4.21%
S	0.11%

Table 7: Composition of MIT wet-oxidation samples. The concentrations are in $\mu\text{g/ml}$ unless otherwise indicated.

SAMPLE ID	Ca	Mg	Mn	P	K	Fe	Ni	Cr	Cl ⁻
Average Detection Limits	0.0021	0.036	0.0022	0.015	0.61	0.012	0.056	0.013	
9-22 #6 (filtered)	0.48	2.3	0.018	6.3	13.5	<.017			360
9-22 #8 (filtered)	0.45	2.9	0.019	10.0	15.8	<.017			500
9-24 #1 (filtered)	0.21	0.18	0.008	6.7	2.0	<.017			<0.1
9-24 #4 (filtered)	0.19	<.036	<.002	3.1	62.0	<.017			57
9-24 #5 (filtered)	0.12	0.36	0.004	8.8	78.0	<.017			110
9-24 #6 (filtered)	0.17	0.41	0.005	14	86	<.017			170
9-24 #7 (filtered)	0.088	0.27	0.004	19	105	<.017			240
10-19 #2 (filtered)	<1.0	0.62	<.002	3.1	74	<.017			120
10-19 #3 (filtered)	<1.0	0.45	<.002	2.4	81	<.017			180
10-19 #4 (filtered)	<1.0	0.26	<.002	0.88	90	<.017			210

Table 7 (continued)

SAMPLE ID	Ca	Mg	Mn	P	K	Fe	Ni	Cr	Cl ⁻
10-19 #2 Suspended Solids Digested	<1.0	0.56	0.035	3.3	47	0.22		0.18	
10-19 #3 Suspended Solids Digested	<1.0	0.38	0.022	3.0	55	0.22		0.088	
10-19 #4 Suspended Solids Digested	<1.0	0.27	0.028	2.1	55	0.22		0.205	
MIT SOLID SAMPLES					UNITS ARE mg/g				
MIT #1 0.274 mg/ml	58	36	0.87	160	<0.0009	17.4	1.2	0.87	
MIT #2 0.1244 mg/ml	22	12	0.39	70	<0.0009	4.8	0.96	0.10	
MIT #3 1.044 mg/ml	55	30	1.2	150	<0.0009	4.9	7.8	0.46	
MIT 10-19 WETOX 0.7282 mg/ml	42	29	0.84	130	30	4.0	1.3	3.83	
MIT 9/11-1 0.2538 mg/ml	134	50	1.3	169	<3.3	7.0		0.41	
MIT 9/22-2 0.1856 mg/ml	91	53	1.3	145	<3.3	12.4		1.81	

4.0 Development of Ion Chromatographic Techniques Using Conventional HPLC (High Performance Liquid Chromatography) Equipment

Waste oxidation and food production research in the CELSS program rely heavily on quantitative inorganic analysis. Atomic absorption spectrometry, and more recently plasma emission spectrometry, provide powerful tools for elemental analysis at the trace levels often of importance in these areas of research. Atomic spectroscopy techniques do, however, have some serious limitations including:

1. They provide no information about the chemical bonding associated with an element; eg., it is possible using special techniques to determine total N using ICPOES but it is not possible to distinguish between NH_4 , NO_3 or NO_2 .
2. Cl^- and F^- ions cannot be determined using commercially available systems, and S is difficult if good precision is required.
3. These techniques do not lend themselves to online real time monitoring; especially in a multielement mode.
4. The sample matrix may interfere.

Ion chromatography, a technique which has gained considerable attention since its introduction by Small,¹ Stevens, and Bauman in 1975, fills many of these gaps while possessing a number of additional potentially useful characteristics. Ion chromatography will not replace atomic spectrometric technique for trace element analysis, but it is another extremely useful tool.

¹ Anal. Chem., 47, 1801 (1975)

Figures 1 and 2 are chromatograms illustrating the ability of ion chromatography to separate some common anions and cations. The retention time of an ion under given chromatographic conditions is useful for identification, and the area under the peak is usually a simple function of concentration. As is characteristic of other chromatographic techniques, separation eliminates most background or matrix effects.

Ion chromatography as introduced by Small et al. involves three steps: (1) separation of the ions of interest by ion exchange chromatography, (2) suppression of the background eluent conductivity by means of a second ion exchange column (suppressor column), and (3) detection by monitoring solution conductivity.

As indicated by Figures 1 and 2, this technique has proven very successful. There are, however, some serious drawbacks including:

1. The suppressor column must be regenerated periodically, thus requiring additional reagents and complicating applications involving online monitoring.
2. The low conductivity of the weak acids formed in the suppressor column during analysis for ions such as silicates, cyanide, or borates makes trace analysis for these ions difficult.
3. The suppressor column tends to broaden and skew the analyte peak, i.e., reduce the chromatographic efficiency. This effect is particularly important if an element specific detector such as ICPOES is interfaced with the IC because maximum emission intensity, i.e., peak height is used for quantitation.

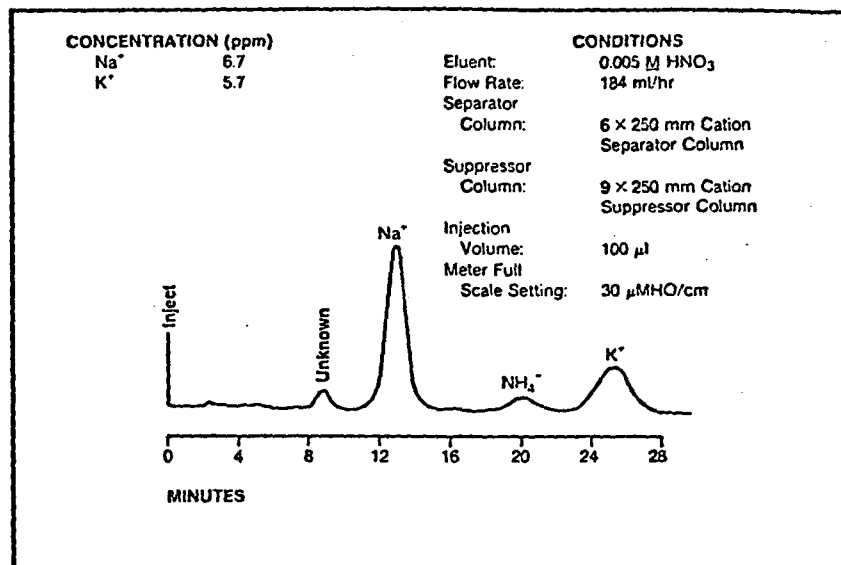


Figure 1. Ion Chromatographic Separation of Simple Monovalent Cations.¹

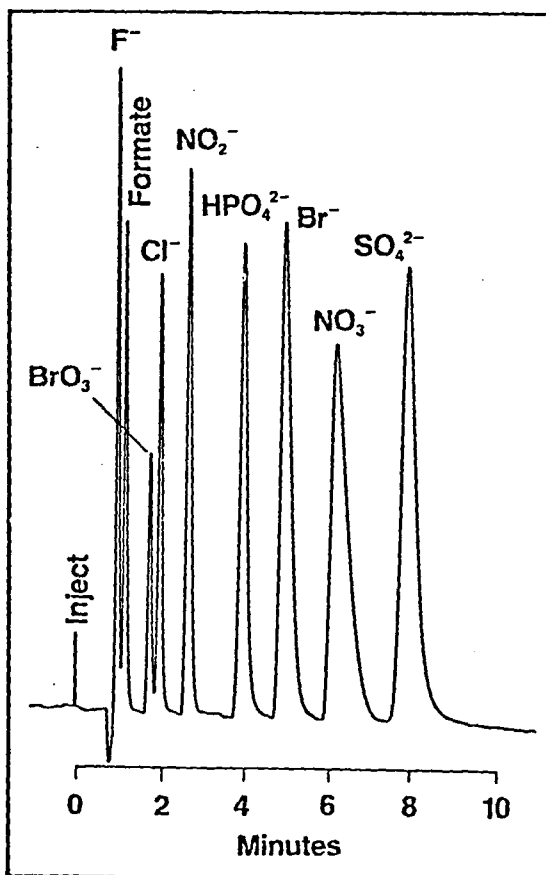


Figure 2. Ion Chromatographic Separation of Simple Anions.¹

¹ From sales literature of the Dionex Corporation

Recently, interest has grown in nonsuppressed IC,¹ i.e., IC without a suppressor column. We have chosen to investigate this approach because of potentially improved performance in online monitoring and coupling with an ICPOES. Figure 3 is a schematic of our system showing the major components. We are currently evaluating the system and anticipate using it to characterize some of the anions in the MIT wet-oxidation samples. In the near future we plan to use the system to investigate nonsuppressed ion chromatography for cation analysis, a little-explored area, the effects of using differential conductivity detection to minimize thermal and background effects, and the interfacing of IC with ICPOES. This latter task is discussed in the next section.

5.0 Investigation of Techniques for Interfacing an Ion Chromatograph with an Inductively Coupled Plasma Optical Emission Spectrometer

The primary function of the interface is the efficient transport of analyte from the liquid stream exiting the ion chromatograph to the inductively coupled plasma. In order for the plasma to convert the analyte solution efficiently to free atoms which can then be excited to give the desired radiation, it is essential that an appropriate aerosol be presented to the plasma. In this context, this necessitates the production of an aerosol with a small mean droplet size, as aerosol particles greater than 10 μm diameter cannot be efficiently desolvated and vaporized by the plasma. In conventional atomic spectrometry, the desired fine aerosol particles are selected from a relatively coarse distribution produced by a pneumatic nebulizer, by means of a spray chamber. The spray chamber acts

¹ Gjerde, D. T. and Fritz, J. S., J. Chromatography 186, 509 (1979).

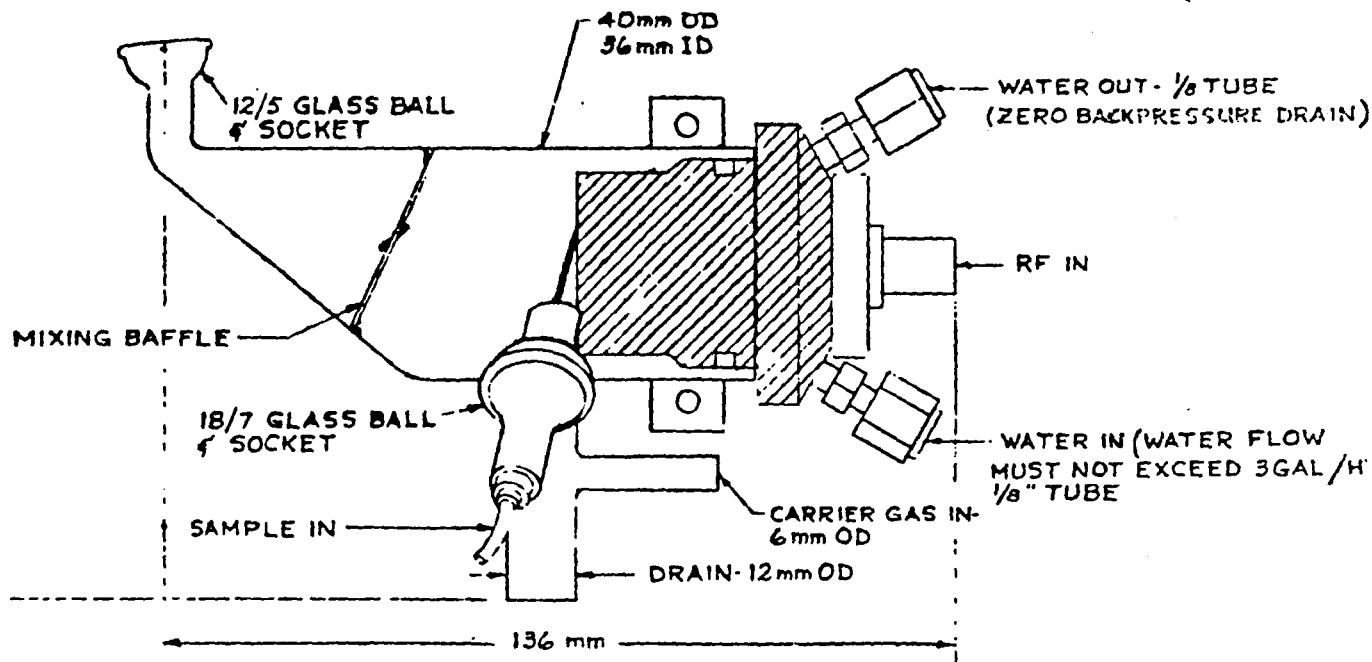


Figure 3

as a filter which discards the larger droplets. Unfortunately, this process acts as a filter which discards the larger droplets. Unfortunately, this process is extremely inefficient, resulting in only 1-2% of the liquid steam entering the nebulizer actually reaching the plasma.

The transport efficiency, ϵ_n , typical of pneumatic nebulizer/spray chamber combinations is unacceptable for IC/ICP interfacing, as the amount of analyte in the sample peak is already low. In order to improve transport efficiency, two ultrasonic nebulizer designs have been tested. One system was a commercially available device (Plasma-Therm) in which the effluent steam from the IC is fed across the face of a piezoelectric crystal, oscillating at 1.4 MHz (Fig. 3). This disrupts the liquid into a fine aerosol spray. The second device, which was constructed especially for this study, but not tested so thoroughly for lack of time, was the Berglund-Liu type of ultrasonic nebulizer. In this device (Fig. 4), liquid effluent from the IC is passed through a narrow (5-20 μm diameter) orifice. The orifice is vibrated by a piezoelectric crystal. By controlling the liquid flow rate and oscillating frequency, typically in the range (200-400 kHz) aerosol of known uniform size can be generated.

5.1 Characterization of Drop Size Distribution and Transport Efficiencies

The Plasma-Therm ultrasonic nebulizer was characterized in terms of the important parameters, transport efficiency and drop size distribution. This was accomplished using published procedures developed in our laboratories^{1,2} and is shown in Fig. 5. The transport efficiency was measured as a function of both liquid flow rate and power to the transducer. The results are shown in Table 8. The direct relationship between RF power and transport efficiency and the flow rate and transport efficiency is clear.

¹ M. S. Cresser and R. F. Browner, Spectrochem. Acta, 35B, 73 (1980).

² D. D. Smith and R. F. Browner, Anal. Chem. 54, 533 (1982).

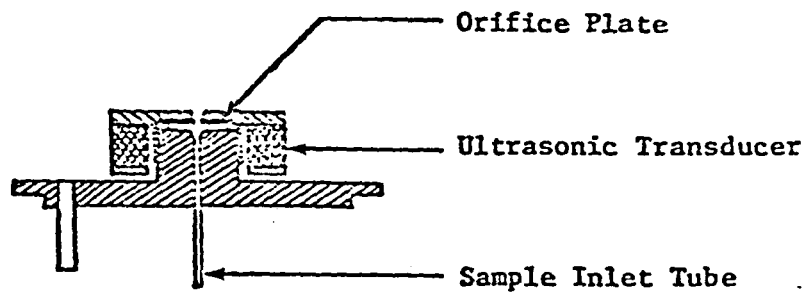


Figure 4. Basic configuration and components of a Berglund-Liu droplet generator.

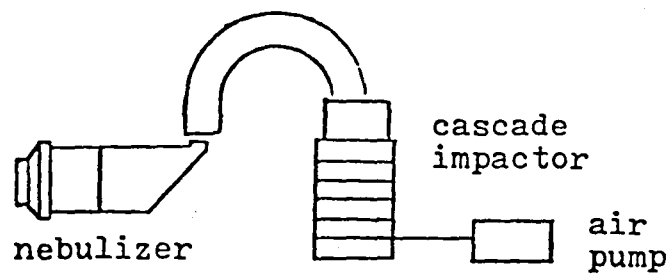


Figure 5

For this system, the lowest flow rate used (0.5 ml/min) and the highest power used (50 W) produced the highest transport efficiency ($\epsilon_n = 36\%$). The influence of power on drop size distribution at a low flow rate is also interesting. Here, the increase in RF power from 20 to 50 W results in an increase in the mass of aerosol contained both in small (1.5 μ m drops) and larger (14 μ m) drops (Fig. 6). In fact the increase in concentration of the larger drops is the most pronounced. The drop size peak showing at approximately 0.3 μ m is probably largely due to evaporation effects.

It can be concluded that with this system, a very high transport efficiency can be obtained at quite low solution flow rates and moderate RF power. These conditions are quite compatible with IC/ICP coupling. The gain in mass transported to the ICP should be approximately 18x, which should result in a substantial benefit in efficiency of transport of analyte peaks contained in the solvent steam for the IC.

The Berglund-Liu type of nebulizer has the potential for producing a higher proportion of droplets (close to 100%) in a suitable size range. Work with this system is currently underway.

RF POWER (W)	FLOW RATE (mL/MIN)		
	0.5	1.0	2.0
20 ± 2	2.4	2.8	1.0
30 ± 4	14	12	4.8
40 ± 4	28	13	11
50 ± 4	36	18	10

TABLE 8
Transport Efficiency

600

-25-

MASS/ΔD
(μg/μm)

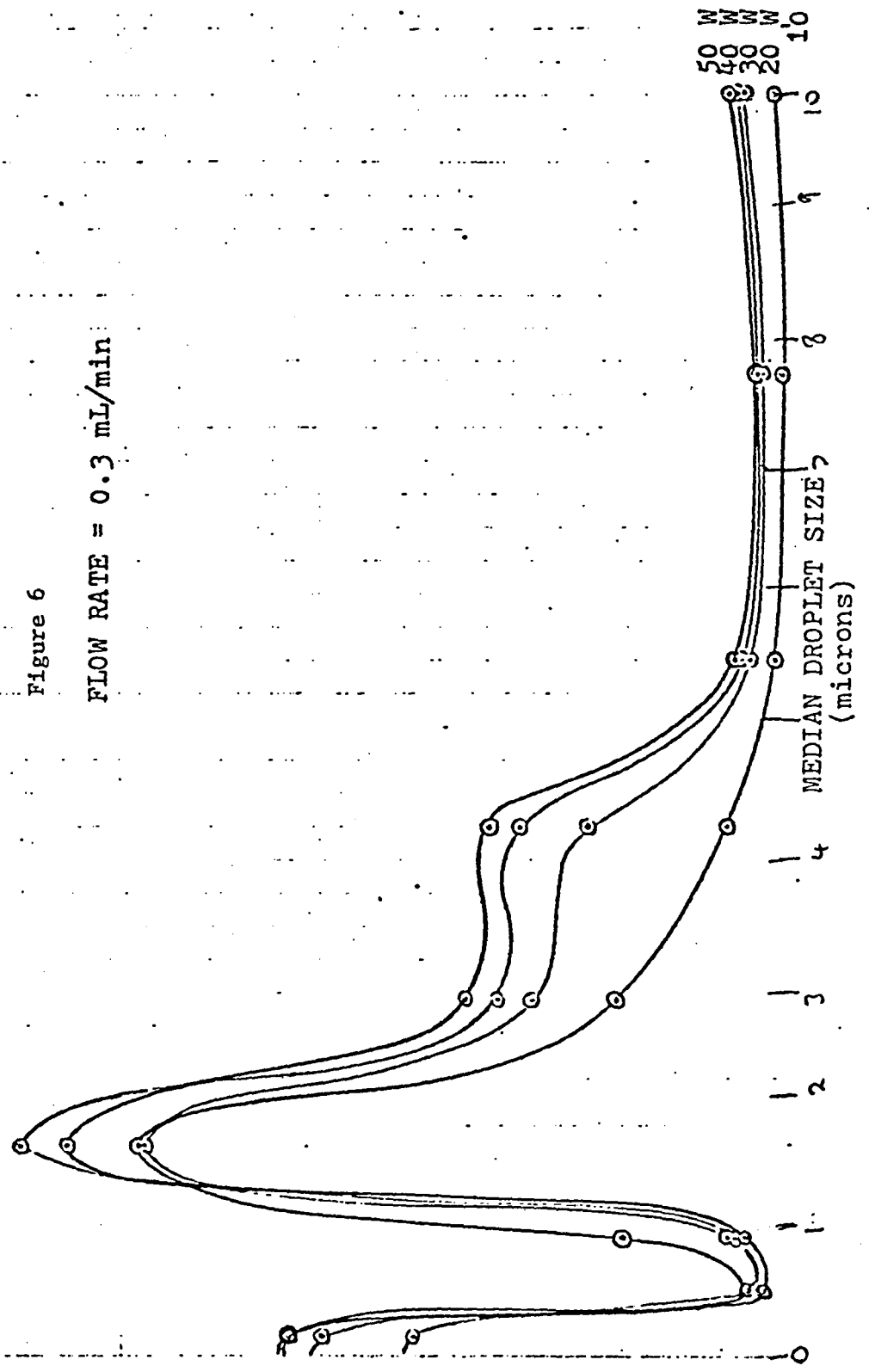


Figure 6

FLOW RATE = 0.3 mL/min

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16. Abstract This report considers the preparation and analysis of standardized waste samples for controlled ecological life support systems (CELSS). Also included are discussions of the analysis of samples from wet oxidation experiments, the development of ion chromatographic techniques utilizing conventional high pressure liquid chromatography (HPLC) equipment, and an investigation of techniques for interfacing an ion chromatograph (IC) with an inductively coupled plasma optical emission spectrometer (ICPOES).			
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