16 M. Palys, M. Bos and W. E. van der Linden, *Anal. Chim. Acta*, 248 (1991) 429.
17 M. Esteban, I. Ruisánchez, M. S. Larrechi and F. X. Rius, submitted for publication.
18 M. Esteban, I. Ruisánchez, M. S. Larrechi and F. X. Rius, submitted for publication.
19 R. Boqué, F. X. Rius and D. L. Massart, submitted for publication.
20 W. Petersen, *Anal. Chim. Acta*, 248 (1991) 77.
21 T. Riley and A. Watson, *Polarography and Other Voltammetric Methods. ACOL Series*, Wiley, Chichester, 1987 (and references therein).
22 A. P. Wade, S. R. Crouch and D. Betteridge, *Trends Anal. Chem.*, 7 (1988) 358.
23 *KES PS 1.4 version*, Software Architecture and Engineering Inc., Arlington, USA, 1990.
24 D. Michie and I. Bratko, *Expert Systems. Automatic Knowledge Acquisition*, Addison-Wesley, Wokingham, 1986.

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trends

Biomolecular tracing through accelerator mass spectrometry

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Accelerator mass spectrometry (AMS) has been developed through the past decade into a sensitive tool for the detection of radio-isotopes, primarily for isotope tracing and chronometry in the earth sciences and archaeology. In the past few years, initial experiments have demonstrated the usefulness of this technique for detection of ^{14}C, ^{26}Al, and ^{41}Ca in biomolecular tracing. We review the advantages of using AMS detection of radio-isotopes in biomedical research.

Introduction

Accelerator mass spectrometry (AMS) using tandem electrostatic (Van de Graaff) accelerators developed as a geochronology tool during the 80's. This development is best recorded in the proceedings of recent ^{14}C conferences [1] and five AMS conferences [2–4]. The suggestion of using AMS for detection of ^{14}C in biomedical studies was first made in 1978 [5], and the possible importance of AMS for tracing ^{26}Al in neurological research was mentioned in 1980 [6]. Possible biomedical applications of AMS were discussed in 1987 [7]. However, active work to fully integrate biomedical procedures with AMS technology started only in 1988 at Lawrence Livermore National Laboratory (LLNL), where biomedical scientists joined the physicists and earth scientists in planning and building the Center for AMS (CAMS). At present, this is the only AMS facility (of 20–30 in the world) in which biomedical measurements are a large fraction of the analysis program. The widespread, and still current, resistance to using AMS for biomedical tracing arises primarily from a belief (and experience) that enhanced radio-isotope levels from labeled compounds find numerous, non-biological, routes through random contaminations to the final sample [8]. While it is necessary to be constantly mindful of this possibility, methods are available to control this contamination, and reports of effective biomedical AMS research first appeared in 1990 [9–14].

Biomolecular tracing with AMS combines detection sensitivity from the low natural backgrounds of radio-isotopes with the efficiency and precision of mass spectrometric measurements. AMS counts individual radio-nuclei, independent of the half life of the isotope, rather than detecting their decay. Thus, small amounts of long-lived radio-isotopes ($t_{1/2}$ = 10–10^7 years) can be used as biological tracers; providing stable working compounds, time-independent measurements, less radioactive waste and minimized chemical and radiation exposures. Table 1 lists the radio-isotopes which are presently being used in biomedical studies through AMS detection, along with isotopes which are under development for biomedical applications, and isotopes which may have a role in future biomedical research. Many of these iso-

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topes can replace short-lived isotopes which are detected through decay counting. The current limits of sensitivity and the final chemical form of the sample are given for the isotopes which have been detected with AMS. The most developed AMS capabilities are for measuring ^{14}C.

Accelerator mass spectrometry

AMS is a type of tandem mass spectrometry in which two mass spectrometers are separated by an accelerator providing molecular dissociation as well as an energy gain (Fig. 1). The equipment currently used for AMS includes one form or another of a tandem electrostatic (Van de Graaff) accelerator. We describe our AMS system, but similar components are found in all systems, whether they use the larger and older accelerators from nuclear physics laboratories or the newer and smaller accelerators built exclusively for AMS [21].

A cesium-sputter (fast atom bombardment) ion source converts a fraction of the atoms in a solid sample to negative ions at ≥30 keV. Most samples have been chemically and physically separated, but one laboratory preserves spatial information of the sample while sputtering [22]. Some elements or molecules do not form negative ions. In particular, the instability of the negative ^{14}N ion removes the principal isobar in carbon measurements. The ions are sorted by a low energy mass spectrometer which selects either a single mass sequentially or a set of defined masses simultaneously [23] for injection into the accelerator beam tube. The increasing positive potential in the beam tube accelerates the negative ion toward the 2–15 MV terminal at the center of the machine. The ions traverse a thin carbon foil or a confined, diffuse gas which strips the outer electrons, producing positive ions and dissociating any molecules. The positive ions are then accelerated toward ground potential. Quadrupole lenses select and focus the ion and charge state of interest from among the distribution of states and ions emitted by the accelerator. The second, high energy, mass spectrometer separates the ions of the rare isotope from the ion beam of a common elemental isotope. This latter ion beam, $^{13}C^{4+}$ in our case of counting $^{14}C^{4+}$, is measured in an off-center Faraday cup. The rare isotope is subjected to a second rigidity (or momentum) selection through another magnet, a velocity (energy) selection through crossed electric and magnetic fields, and finally individual ion identification in a multi-anode gas ionization detector. The accelerator not only removes interfering molecular isobars but also raises ion energies, so that atomic scattering from residual gases is negligible throughout the final beam transport and a nuclear particle detector can be used to identify ions through their specific energy losses. AMS systems are ratio mass spectrometers: the integrated ion beam of the stable isotope

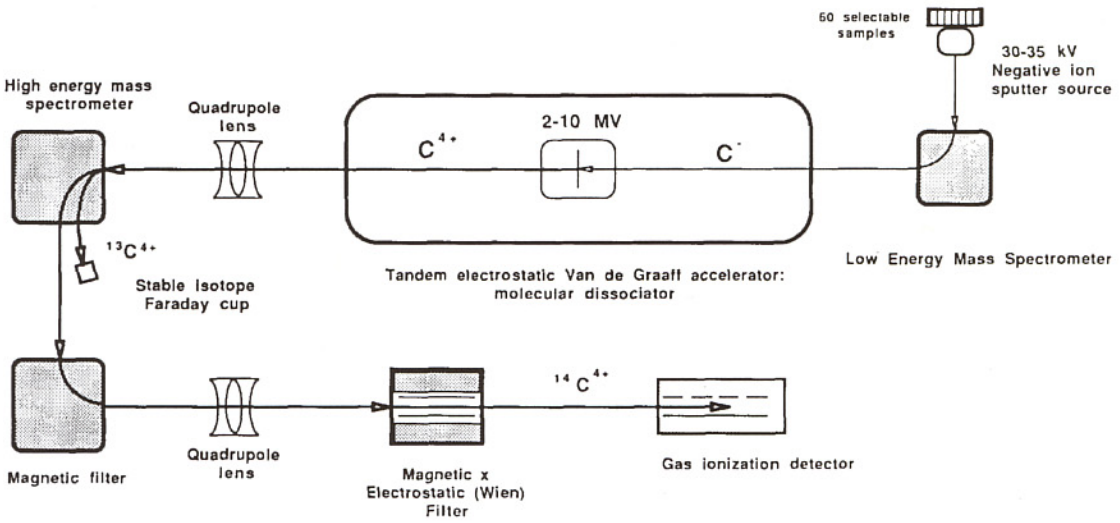

Fig. 1. Schematic of the LLNL accelerator mass spectrometer shows the major components used to obtain part in 10^{15} sensitivity in detecting rare isotopes. This spectrometer, based on a rebuilt tandem Van de Graaff accelerator, is large (accelerator length *ca.* 13.5 m), but smaller systems also exist. The accelerator provides energy gain and dissociates isobaric molecular interferences by stripping two or more electrons from negative ions as they pass through a thin carbon foil or diffuse gas in the center of the accelerator.

corrects the count of the radio-isotopes for variations in accelerator transmission. Although stable, efficient transport is a necessary feature of successful AMS spectrometers, the isotope ratios can not be accepted as absolute. Comparison between standards and secondary standards determines the radio-isotope concentration in an unknown.

AMS samples

The AMS ion source requires approximately 1 mg of sample material in the form of a thermally-conducting low-volatility solid (Table 1). Much of the sputtered sample may be binder or carrier, but AMS measures an isotope ratio and careful inventory must be kept of dilution factors. Non-conducting oxides or very small samples are mixed with metal binders, such as silver. For ^{14}C measurements, the sample is oxidized to CO_2 and reduced to graphite on a cobalt catalyst/binder [24].

The biochemical definition, separation and purification of the sample without introduction of isotopic contamination is important and is often very difficult in laboratories which have a history of radio-isotope use. Our processes for preparation of biomolecular ^{14}C samples will be discussed elsewhere [25]. We use disposable materials and surfaces where possible, and we test reagents before use. In particular, reagents that have complex preparatory chemistry may contain ^{14}C above expected natural levels if they are made or purified in the same equipment used for isotopically labeled forms. Microgram quantities of these materials have only a fraction of radioactive disintegrations per minute more than a surrounding natural matrix. These compounds appear to be unlabeled for scintillation purposes, but we have received 'unlabeled' heterocyclic amines which contain 2.2 μCi of ^{14}C per mole carbon — 30 000 times natural ^{14}C concentrations [8]. The preferred range of final AMS samples in the ion source is between 7 nCi/mole carbon and 700 fCi/mole carbon (1% to 100 times natural levels). Equipment, storage areas and bench tops must also be characterized for ^{14}C levels through AMS before use. Since some long-lived isotopes such as ^{26}Al, ^{36}Cl and ^{41}Ca

TABLE 1. Long-lived isotopes for biomolecular tracing with accelerator mass spectrometry

Element	AMS isotope	Half-life (years)	Scintillation isotope	Half-life (days)	Sensitivity (parts per 10^{15})	Chemical form	Some biomedical applications
Hydrogen[a]	^{3}H	12.3	–	–	?	TiH_2	Biochemical, pharmacological and xenobiotic label
Beryllium[b]	^{10}Be	$1.6 \cdot 10^6$	^{7}Be	53.3	5–10	BeO	Element metabolism and toxicology
Carbon[b]	^{14}C	5730	–	–	3	Graphite	Biochemical, pharmacological and xenobiotic label; clinical diagnostics
Aluminum[b]	^{26}Al	720 000	^{29}Al	0.0045	3–5	Al_2O_3	Element metabolism
Chlorine[b]	^{36}Cl	300 000	^{39}Cl	0.039	5–10	AgCl	Label, intermediary metabolism
Silicon	^{32}Si	130	–	–	$15 \cdot 10^6$ [15]	K_2SiF_6	Bone metabolism
Calcium[a]	^{41}Ca	100 000	^{45}Ca	165	2 [16]	CaH_3, CaF_3	Element metabolism; bone development and disease; cell regulation
Manganese	^{53}Mn	$3.7 \cdot 10^6$	^{54}Mn	312.2	30 000 [17]	Mn_2O_3	Element metabolism
Iron	^{60}Fe	$1.5 \cdot 10^6$	^{55}Fe	978	1000 [18]	Fe	Element metabolism, protein labeling
			^{59}Fe	44.5			
Selenium	^{79}Se	65 000	^{75}Se	119.8	[c]	[c]	Trace element metabolism protein labeling
			^{35}S	87.2			
Technetium	^{97}Tc	$2.6 \cdot 10^6$	$^{95}Tc^m$	61	[c]	[c]	Therapeutics, drug label; human subject research
	^{98}Tc	$4.2 \cdot 10^6$					
	^{99}Tc	213 000					
Iodine[a]	^{129}I	$16 \cdot 10^6$	^{125}I	59.9	10–20 [19,20]	AgI	Thyroid metabolism; immune assays; protein labeling
			^{126}I	13			
			^{131}I	8			

[a] Under development for measurements at CAMS.
[b] Currently measurable at CAMS.
[c] Undeveloped for AMS.

were seldom used previously, laboratories and equipment are less likely to have been contaminated by these isotopes.

Elemental tracers

AMS provides a method to detect long-lived isotopes of elements for which metabolic and toxicological information has been lacking. Aluminum in its elemental form has been present in the human environment for only 100 years. Its effects on and contribution to neurological and renal disorders had been difficult to establish, because aluminum isotopes capable of being counted by decay have half-lives of 6.5 min or less. Instead, complexing reagents along with chromatographic methods and atomic absorption spectroscopy are used to obtain sensitivities of a few μg/l, if the contamination from ubiquitous ^{27}Al is minimized in the laboratory [26]. Gross kinetics of aluminum in rats for periods of 21 days [27] and in a human for periods up to 106 days [28–30] are now studied using AMS detection of ^{26}Al to sensitivities of a few hundred pg/l.

Calcium kinetic experiments, particularly those involving metabolic abnormalities in calcium/bone pools and tissue calcification, may now use the long-lived radio-isotope of calcium, ^{41}Ca, as well as the low-abundance stable isotopes [12]. ^{41}Ca is much cheaper to produce than the purified stable isotopes and provides an inherently more sensitive tracer because it has very low natural abundance. Mass spectrometry is used in multiple source/sink experiments with two or more stable calcium isotopes [31]. AMS could be used in such experiments to detect ^{42,41}Ca with respect to ^{40}Ca.

Table 1 also lists isotopes for which only speculations of elemental tracing exist. Heretofore, there was no usable tracer isotope for chlorine, but quantification of ^{36}Cl is now possible [32]. Beryllium and selenium have isotopes of high specific activity (short half-lives), but experiments previously had to balance detection ease against the toxicity of the elements. AMS could track the low-activity isotopes of these and other necessary elemental micro-nutrients at doses far below toxicities or natural concentrations, which is particularly important in nutrition research with human subjects.

Labeled compounds

The applications of radio-labels are numerous and encompass almost all fields of biological research. We are particularly interested in the role xenobiotics play in initiating mutations (beneficial, benign or detrimental). We use various hosts, from cell lines to rodents and primates, in order to detect the effects of toxins, mutagens, carcinogens, or chemotherapeutics in living systems. Biological effects of these materials, such as genotoxicity, are quantified as functions of the chemical dose to a target tissue. One measure of genotoxicity is obtained by counting the number of DNA adducts (covalently bonded molecules on DNA bases) as a function of the applied chemical dose after exposure to ^{14}C-labeled molecules. Both the dose and the response are quantified by the amount of ^{14}C in the tissue and in the purified DNA, respectively. Previously, limitations in detecting isotope labels forced experiments to be performed at relatively high chemical and radioactive doses. Further, detection of radio-isotopes through counting decays becomes more uncertain toward lower doses. Large uncertainties can arise in the extrapolation of such dose and response data to environmentally relevant doses (Fig. 2). The extrapolations mask the important distinctions among threshold effects or non-linear *versus* linear responses at low doses. Through the sensitivity of AMS, we obtain direct stoichiometric quantification of this biological response, adducts, at tissue concentrations relevant to real human ex-

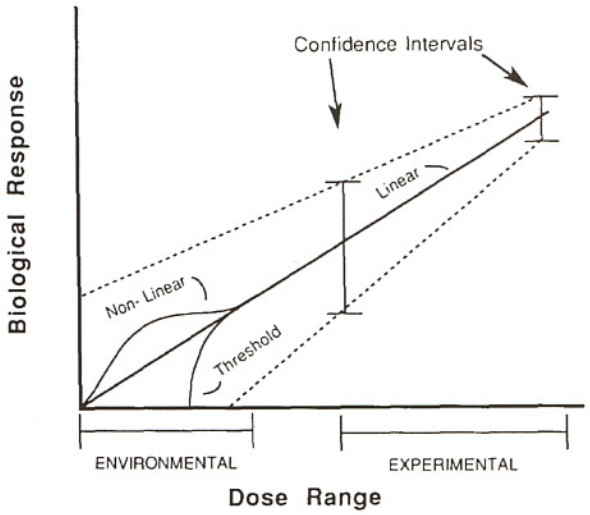

Fig. 2. The biological effect of a compound on a target tissue is related to the dose received by that tissue. Many techniques are limited to high experimental doses because they lack sensitivity in detecting both the low dose delivered to the target and the assayed response. Extrapolations from high-dose data may not predict low-dose effects due to threshold or non-linear responses. AMS provides the sensitivity to measure delivered doses comparable to human exposures and to measure DNA adducts stoichiometrically at these doses.

posure [9]. Radiocarbon is our choice for labeling candidate compounds, since the xenobiotics are usually organic chemicals in which a ^{14}C atom can be incorporated in a non-labile location without causing any change in chemical behavior. Similarly, tritium can be easily incorporated into a wide variety of chemicals, and the value of tritium AMS in quantifying molecular interactions is now being explored [33].

Fig. 3 shows the excess ^{14}C content of murine urine, feces and blood after a single 41 ng/kg dose of the labeled carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), equivalent to the human dose received from 200 g of fried ground beef. We are studying the time history of this chemical in 11 organs, blood, and excretions of this murine host to produce a complete pharmacokinetic model of the distribution of PhIP after a single exposure [34]. In comparison with previous experiments at a 100 mg/kg dose, we find that the PhIP clears the body with a biological halflife of 1.14 h instead of 24 h [35]. In similar studies of carcinogens in primates [36], we show that AMS is sensitive enough to detect labeled adducts on lymphocyte DNA from simple blood extractions, reducing the need of taking complex biopsies from valuable primate models. This sensitivity for testing covalent bonding of a compound to DNA in cultures and murine or primate hosts means that AMS will be a highly sensitive first step in testing new compounds for genotoxicity. Since many cancer chemotherapeutics also act through their genotoxicity in cancerous cells, drug effectiveness could be monitored similarly.

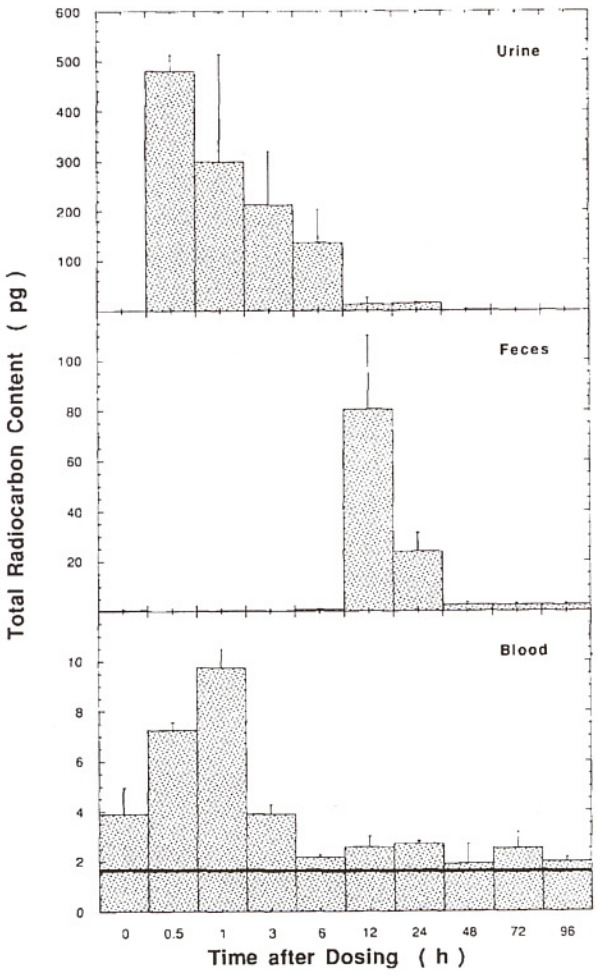

Fig. 3. The amount of ^{14}C detected in the urine, feces and blood of mice is quantified as a function of time after a single exposure to 1 ng of a ^{14}C-labeled carcinogen. The concentration in the blood returns to the natural level, 1.4 pg/g, in 6–12 h. Essentially all of the dose is excreted within 12–24 h, much less than the clearance time found at higher doses where saturation of biochemical processes may occur.

We have also used the *absence* of ^{14}C as a biomarker in the study of dermal hydrocarbon balance in diseased skin [37]. Since AMS is very precise in quantifying ^{14}C concentrations in small samples of natural materials [38], we detect the presence of fossil-derived emollients (petroleum jelly) in separated fractions of dermal lipids which should otherwise contain only contemporary ^{14}C concentrations. Previous research pooled lipids from 10 or more patients [39], but we assay samples from each patient to detect individual varia-

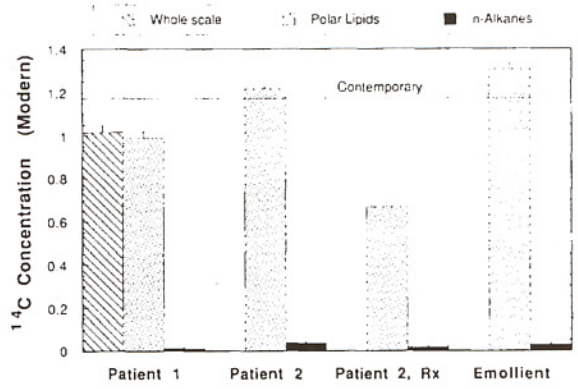

Fig. 4. The ^{14}C concentrations in diseased skin scrapings and in the lipid extractions from the skin of two patients, who ceased their emollient use for 1 month, indicate a possible inclusion of fossil alkanes in the polar lipids. The lipids from one patient were tested during medication as well (R_x). The concentration is measured in units of 'Modern', equal to the concentration of ^{14}C in 1950 A.D. air (6.1 pCi/gram carbon). Contemporary biogenic materials are above Modern due to atmospheric nuclear tests in the early 1960's. The petroleum jelly emollient has recently biogenic polar lipids, but it is primarily made of fossil carbon alkanes.

tions. Fig. 4 displays data from two patients who ceased using the emollients for a month before sampling was performed. The non-polar lipids (alkanes) clearly derive from the petroleum jelly, which is made from fossil fuel carbon. The jelly also contains recent, biogenic polar compounds. There are some indications that fossil components are metabolized into the polar pool and are retained in the unfractionated tissue. We are further fractionating the polar lipid components to see if direct evidence of alkane metabolism can be found.

AMS is not restricted to experiments in which an isotopic label is on a predetermined reactant. Environmental toxins are seldom isotopically labeled when produced, and their concentrations are too small to quantify through any fossil carbon signatures. We are attempting to detect unlabeled chlorinated hydrocarbons by a competitive radioimmunoassay using the chemically exact, labeled ligand as the competitor. Fig. 5 shows a direct binding assay of the ^{14}C-labeled insecticide 1,4,5,6,7,8,8-heptachloro-3*a*,4,-7,7*a*-tetrahydro-4,7-methano-1H-indene (heptachlor). In this assay, hydrophobic losses to the vessel during reaction and volatile losses during the required drying before combustion account for the large fraction of missing heptachlor. These losses are now less than 20%. However, the repeatability of the receptor interaction and the sensitivity of the AMS measurement are shown. Each data point represents the mean of three separate assays, with the uncertainty in the mean being smaller than the data symbol in some cases. The uncertainty rises with dose because the detector cannot distinguish separate counts from samples much greater than 100 Modern. At the lowest dose, AMS quantified 60 ± 8 amol of the 8.5%-labeled compound. Nonspecific binding to the separation media is orders of magnitude lower than these bound fractions. The process of immunoassays using AMS could bring superior sensitivities with simplified extraction chemistries to a number of environmental and medical research problems.

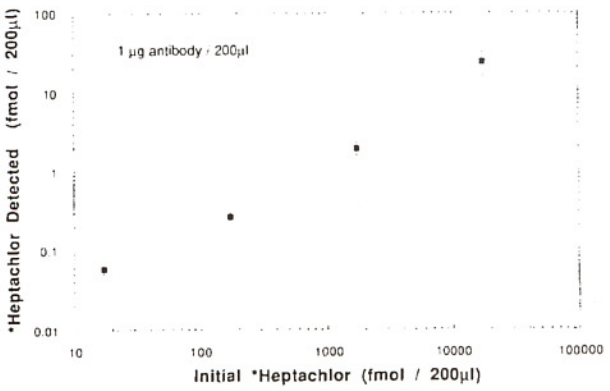

Fig. 5. A direct binding radioimmunoassay for the hydrophobic pesticide, heptachlor, between 10 fmol and 20 pmol demonstrates the precision and sensitivity possible in AMS measurements and the reproducibility in our assay protocols. Each data point has error bars representing the uncertainty in the mean of three independent assays for each concentration of heptachlor. Pulse pile-up in the detector at high concentrations causes the larger uncertainties toward higher doses. AMS can easily quantify tens of amol per mg carbon.

The question of proper units for describing AMS results was one of the first problems to be faced when we began biomedical research with AMS four years ago. The unit 'Modern' is a well defined unit of radiocarbon content which has been used in carbon dating for several decades. It is defined to be 0.95 the radiocarbon activity of an oxalic acid standard from the U.S. NIST (NBS oxalic acid, SRM-4990B). Modern can be shown to be equivalent to $59.0 \cdot 10^9$ ^{14}C atoms per gram of carbon and is an approximation to the content of the atmosphere at 1950, before extensive nuclear testing had begun. We find this unit to be useful in our work, because ^{14}C concentrations so expressed give an immediate sense of relation to current biogenic levels. A sample at 50 000 Modern is a material which must be handled carefully: 20 ng of such material will double the ^{14}C in a mg of contemporary tissue! However, many biomedical researchers are more accustomed to decay-derived units (μCi/mmol, or μCi/mg), as used in this section in order to emphasize our sensitivity (Modern is 6.11 pCi/g).

While ^{14}C provides a long-lived label without chemical modification of most compounds, the technology and chemistry are well developed for labeling proteins, enzymes and pharmaceuticals with other elements. ^{129}I, ^{97,98,99}Tc, ^{79}Se, and ^{60}Fe, with their extremely low specific activities, may be possible labels for drug titration and reactant tracing in human subjects. These long-lived isotopes offer stable chemical compounds over many years, alleviating the problems of normalization and comparison.

Future directions

AMS facilities for specifically biomedical research are a likely outgrowth of present research. Our facility measures a range of isotopes, and has a high throughput of >100 samples during the one-half day per week we make biomedical measurements. This throughput will reduce the per sample measurement cost to less than US$ 50, from the US$ 300–500 cost for archaeological or geological samples at most AMS facilities. Our throughput could increase and cost decrease if samples were delivered ready for measurement. Preparation of these samples could become a routine function of laboratories where the biomedical studies are performed, but the difficulty of avoiding contamination should not be underestimated. For the technology to become widely used, however, more facilities will be required. Since ^{14}C and ^{3}H will be the isotopes most in demand, we and others are researching designs of smaller, isotopically limited AMS machines [21,33,40]. We expect that a regional 'Center' or two might be established initially. With the further development of AMS in biomedical research, individual institutions might invest in dedicated machines, leading to eventual clinical diagnostic use.

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References

1 International Radiocarbon Conference; 11th: *Radiocarbon*, 25,2 (1983) 795 pp.; 12th: *Radiocarbon*, 28, 2A (1986) 804 pp.; 13th: *Radiocarbon*, 31, 3 (1989) 1082 pp.; 14th: *Radiocarbon*, 33,2 (1991) and 34 (in Press).
2 H.E. Gove (Editor), *Proceedings of the First Conf. on Radiocarbon Dating with Accelerators*, University of Rochester, Rochester, NY, 1978, 401 pp.
3 W. Kutschera (Editor), *Proceedings of the Symposium on Accelerator Mass Spectrometry*, Argonne Natl. Lab., Argonne, 1981, 501 pp.
4 International Conference on Accelerator Mass Spectrometry; 3rd: *Nucl. Inst. Meth. Phys. Res.*, B5, 2 (1984) 448 pp.; 4th: *Nucl. Inst. Meth. Phys. Res.*, B29, 1–2 (1987) 455 pp.; 5th: *Nucl. Inst. Meth. Phys. Res.*, B52, 3–4 (1990) 630 pp.
5 J. Keilson and C. Waterhouse, in H.E. Gove (Editor), *Proceedings of the First Conf. on Radiocarbon Dating with Accelerators*, University of Rochester, Rochester, NY, 1978, p. 391.
6 A.E. Litherland, *Ann. Rev. Nucl. Part. Sci.*, 30 (1980) 437–473.
7 D. Elmore, *Biol. Trace Elem. Res.*, 12 (1987) 231–245.
8 J.S. Vogel, J.R. Southon and D.E. Nelson, *Radiocarbon*, 32 (1990) 81–83, discussion: 84–86.
9 K.W. Turteltaub, J.S. Felton, B.L. Gledhill, J.S. Vogel, J.R. Southon, M.W. Caffee, R.C. Finkel, D.E. Nelson, I.D. Proctor and J.C. Davis, *Proc. Natl. Acad. Sci. USA*, 87 (1990) 5288–5292.
10 J.S. Felton, K.W. Turteltaub, J.S. Vogel, R. Balhorn, B.L. Gledhill, J.R. Southon, M.W. Caffee, R.C. Finkel, D.E. Nelson, I.D. Proctor and J.C. Davis, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 517–513.
11 J.S. Vogel, K.W. Turteltaub, J.S. Felton, B.L. Gledhill, D.E. Nelson, J.R. Southon, I.D. Proctor and J.C. Davis, *Nucl. Instrum. Methods Phys. Res., B*, 52 (1990) 524–530.
12 D. Elmore, M.H. Bhattacharyya, N. Sacco-Gibson and D.P. Peterson, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 531–535.
13 O. Meirav, R.A.L. Sutton, D. Fink, R. Middleton, J. Klein, V.R. Walker, A. Halabe, D. Vetterli and R.R. Johnson, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 536–539.
14 J. Barker, J.P. Day, T.W. Aitken, T.R. Charlesworth, R.C. Cunningham, P.V. Drumm, J.S. Lilley, G.W.A. Newton and M.J. Smithson, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 540–543.
15 H.J. Hoffman, G. Bonani, M. Suter, W. Wölfli, D. Zimmerman and H.R. von Gunten, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 544–551.
16 D. Fink, J. Klein and R. Middleton, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 572–581.
17 G. Korschinek, H. Morinaga, E. Nolte, E. Preisenberger, U. Ratzinger, P. Dragovitsch and S. Vogt, *Nucl. Instrum. Methods Phys. Res. B*, 29 (1987) 67–71.
18 W. Kutschera, P.J. Billquist, D. Frekers, W. Henning, K.J. Jensen, M. Xiuzeng, R. Pardo, M. Paul, K.E. Rehm, R. Smither, J.L. Yntema and L.F. Mausner, *Nucl. Instrum. Methods Phys. Res. B*, 5 (1984) 430–435.
19 L.R. Kilius, A.E. Litherland, J.C. Rucklidge and N. Baba, *Appl. Radiat. Isot. Part A*, 43 (1992) 279–287.
20 P.W. Kubik, R.T.D. Sharma, S. Teng, S. Tullai-Fitzpatrick, S. Datar, U. Fehn, H.E. Gove and D. Elmore, *Nucl. Instrum. Methods Phys. Res. B*, 56/57 (1991) 238–241.
21 K.H. Purser, T.H. Smick and P.K. Purser, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 263–268.
22 S.P.T.H. Freeman, C.R. Bronk and R.E.M. Hedges, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 405–409.
23 J.R. Southon, D.E. Nelson and J.S. Vogel, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 370–374.
24 J.S. Vogel, *Radiocarbon*, 34 (1992) in Press.
25 J.S. Vogel *et al.*, manuscript in preparation.
26 E. Kaneko, H. Hoshino, T. Yotsuyanagi, N. Gunji, M. Sato, T. Kikuta and M. Yusa, *Anal. Chem.*, 63 (1991) 2219–2222.
27 O. Meirav, R.A.L. Sutton, D. Fink, R. Middleton, J. Klein, V.R. Walker, A. Halabe, D. Vetterli and R.R.

Johnson, *Am. J. Physiol.*, 260 (1991) F466–F469.
28 J.P. Day, J. Barker, L.J.A. Evans, J. Perks, P.J. Seabright, P. Ackerill, J.S. Lilley, P.V. Drumm and G.W.A. Newton, *The Lancet*, 337 (1991) 1345.
29 J.P. Day, J. Barker, P.V. Drumm, J.S. Lilley and G.W.A. Newton, *J. Anal. At. Spectrom.*, (1992) in Press.
30 J. Barker, J.P. Day, N.D. Priest, P.V. Drumm, J.S. Lilley and G.W.A. Newton, *Nucl. Instrum. Methods Phys. Res.*, (1992) in Press.
31 A.L. Yergey, S.A. Abrams, N.E. Vieira, R. Eastell, L.S. Hillman and D.G. Covell, *Can. J. Physiol. Pharmacol.*, 68 (1990) 973–976.
32 T. Straume, R.C. Finkel, D. Eddy, P.W. Kubik, H.E. Gove, P. Sharma, S. Fujita and M. Hoshi, *Nucl. Instrum. Methods Phys. Res. B*, 52 (1990) 552–556.
33 M.L. Roberts, J.R. Southon, J.C. Davis, I.D. Proctor and D.E. Nelson, *Nucl. Instrum. Methods Phys. Res. B*, 56/57 (1991) 882–885.
34 K.W. Turteltaub, J.S. Vogel, C.E. Frantz and N.H. Shen, *Cancer Res.*, submitted for publication.
35 K.W. Turteltaub, J.S. Vogel, C. Frantz, M. Buonarati and J.S. Felton, *Environ Health Perspect*, (1992) in Press.
36 K.W. Turteltaub, J.S. Felton, J.S. Vogel, E.G. Snyderwine, S.S. Thorgeirsson, R.H. Adamson, B.L. Gledhill and J.C. Davis, *The Toxicologist*, 11 (1991) 131 (abstract only).
37 M.L. Williams, J.S. Vogel, R. Ghadially, B. Brown and P.M. Elias, *Arch. Dermatol.*, (1992) in Press.
38 J.S. Vogel, D.E. Nelson and J.R. Southon, *Radiocarbon*, 31 (1989) 145–149.
39 J.T. Bortz, P.W. Wertz and D.T. Downing, *J. Invest. Dermatol.*, 93 (1989) 723–727.
40 K.H. Purser and H.E. Gove, *Trans. Am. Nucl. Soc.*, 62 (1990) 10–11.

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Solid-phase reactors in flow injection analysis

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The use of a solid-phase reactor coupled on-line to a flow injection manifold can significantly expand the potential of flow injection analysis by enhancing such basic analytical parameters as sensitivity and selectivity, and allowing implementation of specific reactions. Typically, solid reactors involve chemical reactions (whether enzymatic, immunoassay, ion-exchange or redox) or act as sorbent extractants or reagent releasers. These aspects and major trends in their use in conjunction with flow injection manifolds are examined here.

Introduction

After 18 years of continuous growth flow injection analysis (FIA) can be considered to be at the summit of its development phase as an enhancer of analytical features. This enhancement has been achieved through a number of effects, *e.g.*: The higher available selectivity of flow injection methods compared to their manual and automated counterparts is a result of the inherent kinetic character of this technique, and can be further increased in different ways [1]. The great versatility of this technique has fostered the development of a number of ways of altering sensitivity, which can be either increased (stopped-flow, iterative passage of the plug through the detection point, use of solid reagents or micellar media) or decreased (sampling zones, dilution or pseudo-dilution, changes in the flow-rate or sample volume) at will [2].

The degree of automation made possible by a flow injection manifold ranges from that of a basic manual system in which only the progression of the analytical reaction is automated to that of a fully automated analyzer controlled via a microcomputer through passive and active interfaces. By its very nature FIA lends itself readily to miniaturization, a universal trend in analytical chemistry today, which has so far resulted in such modes as capillary FIA [3], integrated microconduits [4] and integrated reaction (retention)–detection [5] systems.

There are other aspects of flow injection development that also testify to the maturity attained by this technique, namely the possibility of directly introducing solid samples into a manifold [6] and

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