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PRODUCTION AND USE OF STABLE ISOTOPES IN FRANCE

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Production and use of stable isotopes in France

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Abstract

This paper can not cover the field of production and use of stable isotopes in France exhaustively within six pages. We have chosen to concentrate on highlights of the subject and on recent work, and to give references for further reading.

1. INTRODUCTION

One of us (E.R.) has given a bibliography of relevant work up to the beginning of 1990, [1] : this paper borrows much of its material to a general study of stable isotope applications made under the direction of R. Létolle, [2].

As the use of stable isotopes is often confronted with limitations due to analytical problems, developments in sampling and measuring techniques that open new possibilities have been given special attention.

2. STABLE ISOTOPE PRODUCTION IN FRANCE

Separated isotopes of Uranium, Lithium and Boron are produced for the nuclear industry by COGEMA, in installations that it is not relevant to describe here.

Tritium is produced at the Laue-Langevin institute (ILL) in Grenoble by the isotope separation process adopted later for the Darlington plant. The capacity of the plant is about 10 grams per year.

Since the N-15 facility production in Toulouse was shut down no other regular separation of isotopes is in operation. However research goes on in several directions : At Pierrelatte ion exchange is investigated for the production of boron-10 and N-15. The process appears competitive for low enrichments. Laser techniques that are under intensive investigation for uranium (AVLIS process) are also studied for other elements (Gadolinium etc ...). But ion Cyclotron Resonance is the technique to which most efforts are devoted by CEA. The motivation is that whereas it can be used to separate small quantities of any isotope, very easily, except perhaps gaseous ones, it also has the potential to produce kilogram amounts at costs that may

be competitive, even of isotopes of heavy elements. The ERIC facility has produced Cr-50, Ca-48, Cd-110, Ba-132, Ni-58, at various concentrations and in small amounts [3].

Apart from miscelleanous needs of physicists that concern either small quantities, or, in contradistinction, large amounts, (for instance of Ni-58 or Cr-50) that are too costly to produce, french consumption of separated isotopes is of the order of a kilogram of C-13, or of N-15, and of several liters of $H_2{}^{18}O$ at enrichments, in every case, ranging from a few percent to 100 %.

Deuterium, as heavy water, is used by the ton. This arises from the fact that like most isotopically enriched "raw" material D_2O is not used as such but to prepare organic deuterated molecules. The main user is the EURISO-TOP company, a new subsidiary of CEA. It produces deuterated solvents, principally deuterated chloroform and dimethylsulfoxide, by the hundreds of kilograms, covering french and a large part of European needs of solvents for NMR. Other solvents, tetrahydrofuran etc ... are also produced. Other labelled molecules are synthetized in various laboratories, among which the "Service des molécules Marquées" of CEA at Saclay, that while devoting its main effort to the production of radioactive molecules, produces a number of stabily labelled ones. EURISO-TOP commercialises such productions and encourages new ones when a demand exists. The department of biology, at Saclay produces uniformly labelled molecules by biological means. Chemical synthesis can, of course, be used to label specific sites.

3. USES OF STABLE ISOTOPES

Foremost applications are in the pharmacological and medical research fields. However studies on natural substances, especially of course in France investigations on wines, contributions to environmental research are of growing importance, and recently isotopes have been shown to be useful in investigating deterioration of concrete under the action of atmospheric CO_2.

3.1. Medical and Pharmacological Research

The straightforward use of isotopes as tracers remains a powerful tool. When investigating the growth of cancerous tumors the question that arises is whether tumors incorporate proteins in priority to normal tissue. Feeding patients with a diet incorporating N-15 labelled glycine showed that concerous tissues synthesize proteins significantly faster than normal ones [4-5]. Research is progressing with C-13 because of better analytical instrumentation in that case.

It was found some years ago that populations in developing countries, and even in industrialized areas, suffer from iron deficiency. Hence the need to study how iron from food is assimilated. After pioneering investigations using radioactive iron it was mandatory to turn to stable isotopes. Fe-58 and Fe-57 enriched to 60-80 % are fed to healthy male adults. Absorption of iron from vegetables has been evaluated to 1-5 % of the iron in the food, and that from meat to 10-25 %. Analysis is made by thermalionization mass spectrometry [6].

Investigations on various phases of the development of diabetes, or on the effect of ketones or of insulin on glucose utilization by the body involve the use of labelled glucose [7-9]. In order to cut the amount and the cost of C-13 needed for one bolus a new gas chromatograph-mass spectrometer technique

is employed. It consists of interfacing a furnace between a capillary gas chromatograph and an isotope ratio mass spectrometer. Though derivatization of glucose is necessary to analyse glucose, and leads to smaller C-13 enrichments at the measuring end, 10 nmol samples of glucose suffice for a determination, i.e. 10 to 100 times less than by previous techniques [10].

Many papers deal with the metabolism of glutamine, and with leucine as its precursor. Glutamine is the main nitrogen carrier in the body [11]. In order to study the oxidation of leucine by breath tests, after giving C-13 labelled leucine or acid sodium carbonate to a patient, a new technique has been developed. Its aim is to avoid the necessity for the patient to blow into a sampling device such as a Douglas bag. This enables tests on infants, on sleeping patients or very feeble ones, even on comatose persons. The device consists of a ventilated canopy. Its is ventilated to limit the CO_2 concentration of air inhaled by the patient. Expired air is thus diluted, but the technique has been carefully validated [12].

In pharmacological research studies of metabolic processes are numerous. They are only valid when no isotope effect is observed, because especially when substituting D for H, very large kinetic effects could occur leading to wrong conclusions respective to reaction rates of ordinary molecules [13].

But isotope effects are useful in the study of reaction mechanisms : due to the large difference in binding energies of carbon between D and H, when rupture of a given C-H bond is involved in a reaction, isotopic labelling of this bond will cause a difference in reaction rates to be observed.

By labelling successively each C-H bond of a molecule one can thus establish which are the active ones. The binding sites of caffein to human serum albumin have been identified by this technique [14].

When C-13 is used as tracer a considerable improvement in its use for metabolic studies is introduced by the possibility of on line analysis. The "Dual inlet dynamic interface" developed in Lyon enables to measure mass spectrometrically, on line, the isotopic composition of CO_2 either from expired air or from various gases or liquids such as alcohols [15].

The simultaneous use of two different isotopes helps to solve difficult problems. Ketone bodies, acetoacetate (AcAc) and -hydroxybutyrate (-OHB) can partially replace glucose in the energy metabolism of the fasting brain. It is important to establish de novo synthesis and irreversible disposal rates for each ketone. A difficulty arises when using tracer techniques for that purpose : interconversion of the two ketones takes place, but complete isotopic equilibrium is not obtained. Therefore using one labelled ketone only does not even enable calculation of rates for that particular species. Labelling AcAc with C-13 and -OHB with D enables calculation of rates for both ketones and, in addition, interconversion rates. Measurements are made from blood samples by GCMS [16].

The previous applications all make use of mass spectrometry for isotopic analysis. Progress in NMR allows the identification of labelled sites. In addition detection limits for C-13 are sufficently low to enable analysis to be performed in vivo and in situ on large animals and on men. Formation of glycogen from glucose is a typical example. Glucose is labelled on carbon 1. By incorporating glucose into glycogen C-13 could only appear on carbon 1. NMR shows that all carbons (one to six) are equally labelled. This proves that synthesis occurs in an indirect way through triosephosphates [17].

An experiment on a baboon requires 3 grams of glucose labelled at 99 % in C-13 on carbon 1, an experiment on man 10 grams.

3.2. Studies of natural substances

It is well known that plants fractionnate isotopes, and the fact that cane sugar is richer in C-13 than beetroot sugar has long been established and put to use either to use cane sugar as a naturally labelled material in countries where the usual sweetener is beetroot sugar, or to prevent the fraud consisting of selling one of the sugars in the stead of the other one.

NMR enables making site specific isotopic analysis, at natural abundances, of C-13 and of Deuterium. This has been applied, using a 9.4 Tesla spectrometer, to determine the influence of environmental and technical parameters on the deuterium distribution in wine products. A purpose of this research was to estalish a method to detect fraudulent chaptalization of wines, i.e. addition of sugar in order to enhance the alcoholic content.

Up to eight isotope ratios may be determined for the water and ethanol species of a wine. We shall consider specially (D/H)I and (D/H)II representing isotope ratios of CH_2DCH_2OH and CH_3CHDOH. (D/H)I and (D/H)II have different values and vary in a different way with environmental conditions and water content. Other isotope ratios vary in a similar fashion ; a principal component analysis conducted on several hundred wine samples, characterized by five isotope ratios, revealed differences linked to the climates of the countries of production. A subsequent factorial discriminant analysis assigned the different samples to previously defined climatic groups.

An example shows the sensitivity of the method in the case of sugar addition. A vineyard producing 6000 l/hectare of wine at an alcoholic grade of 10 % v/v produces 600 l of pure ethanol. The addition of only 20 kg of beet sucrose to the production of one hectare would decrease the (D/H)I ratio by 0.2 ppm and thus be detectable, though it would correspond to the addition of only 1.3 l of beet ethanol to the 600 "natural" ones [18].

The technique can be applied to natural flavouring agents, to perfumes etc ... It is also used to determine site specific liquid-vapour isotopic fractionation parameters : in the case of ethanol inverse isotope effects are exhibited by the two C-13 isotopomers and by the monodeuterated methyl and methylene isotopomers, the hydroxylic isotopomer presenting the normal effect (H/D greater in the vapour phase) [19-20].

3.3. Environmental studies

N-15 has been used to study the efficiency of nitrification inhibitors, the purpose of their usage being to prevent pollution due to the conversion of fertilizers to nitric acid or nitrates.

Ammonium sulphate was used as fertilizer together with dicyandiamide as inhibitor (DCD) over a one year period. DCD keeps the ammonium quantity present constant for six months, provided the temperature of the soil does not exceed 20 °C, above which DCD decomposes. $(NH_4)_2 SO_4$ was labelled at a 31 % enrichment in N-15, and it was found that in the presence of DCD labelled ammonium dissapears faster than total ammonium, indicating that organic matter of soils undergoes ammonification.

In DCD absence the amount of N-15 oxidised under the nitric form levels off while total nitric acid keeps increasing showing a continuing mineralisation of organic matter [21-22].

Recently fertilizers at a low C-13 enrichment have been used to study fertilizers over large areas [23].

3.4. *Studies of carbonatation processes in concretes*

Carbon 13 and Oxygen 18 mass spectrometry is used to establish the mecanism of carbonate precipitation at high Ph. At Ph greater than 10 when grains of cement (portlandite) are surrounded by a film of water, atmospheric CO_2 precipitates without exchanging with the water, as shown by O-18 analysis. If CO_2 is dissolved in water with which it exchanges secondary calcite is then precipitated at a different isotopic composition from the total inorganic dissolved carbon.

At a Ph less than 8 CO_2 always exchanges with water. Experiments were conducted on pure cement species either in controlled systems or in the open.

Isotopic composition of carbonates in concrete makes it possible to distinguish the various phases especially if a limestone filler exists. This enables to establish proportions of carbonates in concretes coming from CO_2, carried by rain or from the filler, or from CO_2 dissolved in water in contact with the concrete.

Proportions varying from 100 %, from rain water CO_2 in the case of a blockhaus built in Normandy, to 66 % CO_2 from the filler in a bridge near Paris have been reported [24-25].

4. ANALYTICAL INSTRUMENTATION

As efforts to adapt measuring techniques in oder to solve specific problems have shown, instrumentation is a key to the development of uses of stable isotopes and indirectly to that of their production.

In addition to the devices already mentioned instruments have been built for direct measurement of Deuterium in blood by infrared absorption, to enable determinations in vivo and in situ [26].

Commercial equipments manufactured in France include quadrupole mass spectrometers and GCMS systems [27], optical, non dispersive and simple, spectrometers for N-15 analysis mostly for agricultural research, [28] and imaging SIMS spectrometers [29].

The latter instruments, constantly improved by Pr. Slodzian, open a vast field of isotopic studies specially of diffusion in solids.

5. MISCELLANEOUS

Many applications currently under study must be omitted in this space limited review. We would just like to add isotopic ion implantation experiments carried out at Laboratoire Bernas at Orsay either to simulate organic synthesis in space or in view of diffusion studies.

6. CONCLUSION

Whereas a variety of applications should lead to a continuous, but propably slow, increase in the use of stable isotopes in France, progresses in NMR techniques and in vivo and in situ determinations for medical

diagnostics may lead to a rapid development of production of C-13 and perhaps D labelled molecules.

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