Introduction into stable isotopes

Stable isotopes in ecology and ecosystem research

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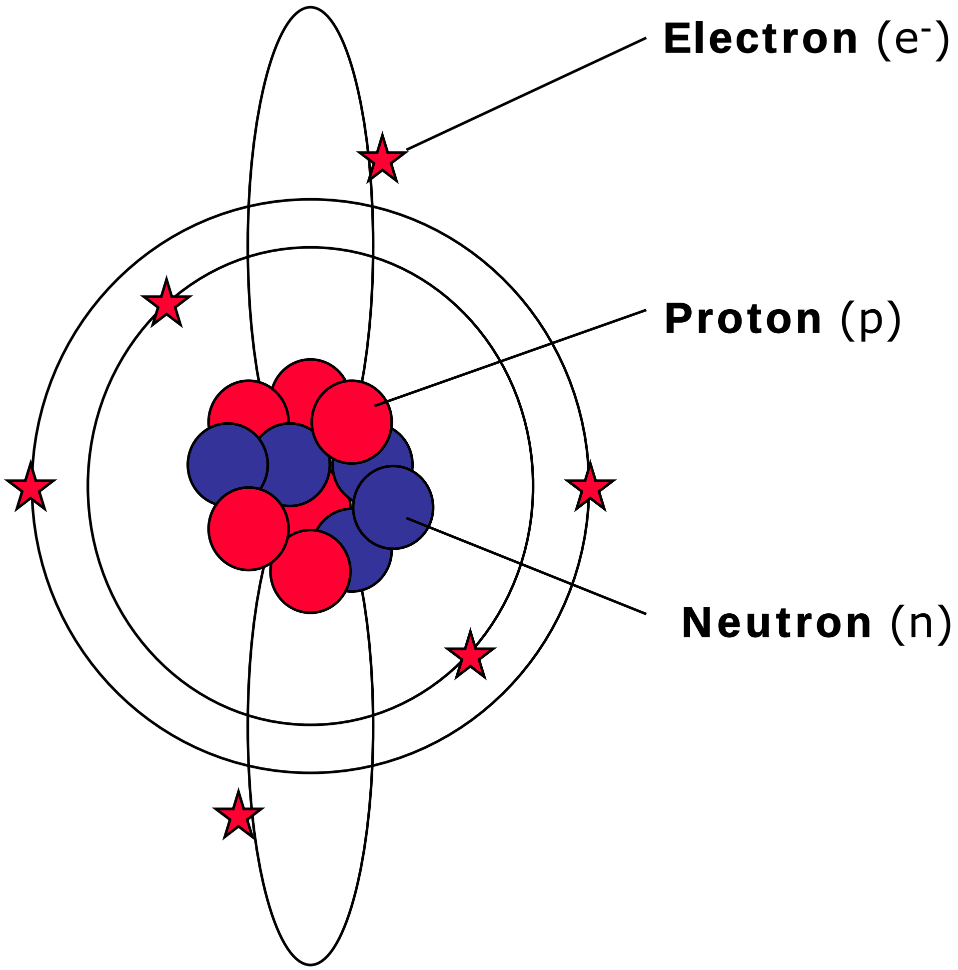
IRMS Delta^{PLUS} (Finnigan MAT)

Elements and isotopes



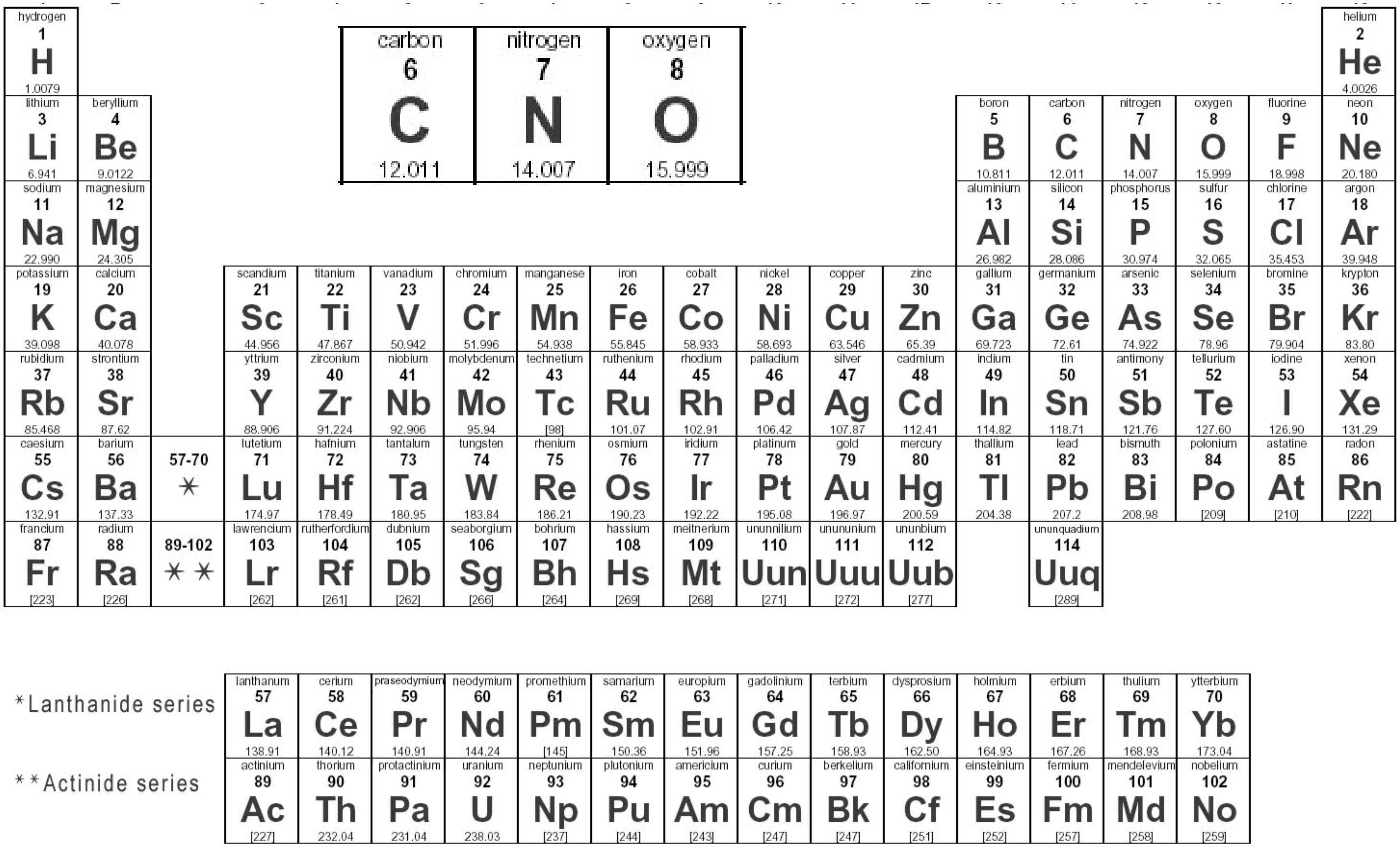
Definition of a chemical element

Atomic number
6 p, 6 e^- -> $_6C$

Definition of an isotope

Mass number
6 p -> $_6C$
6 n -> ^{12}C
7 n -> ^{13}C
8 n -> ^{14}C

Periodic table of elements



carbon 6 **C** 12.011	nitrogen 7 **N** 14.007	oxygen 8 **O** 15.999

hydrogen 1 **H** 1.0079																		helium 2 **He** 4.0026
lithium 3 **Li** 6.941	beryllium 4 **Be** 9.0122												boron 5 **B** 10.811	carbon 6 **C** 12.011	nitrogen 7 **N** 14.007	oxygen 8 **O** 15.999	fluorine 9 **F** 18.998	neon 10 **Ne** 20.180
sodium 11 **Na** 22.990	magnesium 12 **Mg** 24.305												aluminium 13 **Al** 26.982	silicon 14 **Si** 28.086	phosphorus 15 **P** 30.974	sulfur 16 **S** 32.065	chlorine 17 **Cl** 35.453	argon 18 **Ar** 39.948
potassium 19 **K** 39.098	calcium 20 **Ca** 40.078		scandium 21 **Sc** 44.956	titanium 22 **Ti** 47.867	vanadium 23 **V** 50.942	chromium 24 **Cr** 51.996	manganese 25 **Mn** 54.938	iron 26 **Fe** 55.845	cobalt 27 **Co** 58.933	nickel 28 **Ni** 58.693	copper 29 **Cu** 63.546	zinc 30 **Zn** 65.39	gallium 31 **Ga** 69.723	germanium 32 **Ge** 72.61	arsenic 33 **As** 74.922	selenium 34 **Se** 78.96	bromine 35 **Br** 79.904	krypton 36 **Kr** 83.80
rubidium 37 **Rb** 85.468	strontium 38 **Sr** 87.62		yttrium 39 **Y** 88.906	zirconium 40 **Zr** 91.224	niobium 41 **Nb** 92.906	molybdenum 42 **Mo** 95.94	technetium 43 **Tc** [98]	ruthenium 44 **Ru** 101.07	rhodium 45 **Rh** 102.91	palladium 46 **Pd** 106.42	silver 47 **Ag** 107.87	cadmium 48 **Cd** 112.41	indium 49 **In** 114.82	tin 50 **Sn** 118.71	antimony 51 **Sb** 121.76	tellurium 52 **Te** 127.60	iodine 53 **I** 126.90	xenon 54 **Xe** 131.29
caesium 55 **Cs** 132.91	barium 56 **Ba** 137.33	57-70 *	lutetium 71 **Lu** 174.97	hafnium 72 **Hf** 178.49	tantalum 73 **Ta** 180.95	tungsten 74 **W** 183.84	rhenium 75 **Re** 186.21	osmium 76 **Os** 190.23	iridium 77 **Ir** 192.22	platinum 78 **Pt** 195.08	gold 79 **Au** 196.97	mercury 80 **Hg** 200.59	thallium 81 **Tl** 204.38	lead 82 **Pb** 207.2	bismuth 83 **Bi** 208.98	polonium 84 **Po** [209]	astatine 85 **At** [210]	radon 86 **Rn** [222]
francium 87 **Fr** [223]	radium 88 **Ra** [226]	89-102 * *	lawrencium 103 **Lr** [262]	rutherfordium 104 **Rf** [261]	dubnium 105 **Db** [262]	seaborgium 106 **Sg** [266]	bohrium 107 **Bh** [264]	hassium 108 **Hs** [269]	meitnerium 109 **Mt** [268]	ununnilium 110 **Uun** [271]	unununium 111 **Uuu** [272]	ununbium 112 **Uub** [277]		ununquadium 114 **Uuq** [289]				

*Lanthanide series	lanthanum 57 **La** 138.91	cerium 58 **Ce** 140.12	praseodymium 59 **Pr** 140.91	neodymium 60 **Nd** 144.24	promethium 61 **Pm** [145]	samarium 62 **Sm** 150.36	europium 63 **Eu** 151.96	gadolinium 64 **Gd** 157.25	terbium 65 **Tb** 158.93	dysprosium 66 **Dy** 162.50	holmium 67 **Ho** 164.93	erbium 68 **Er** 167.26	thulium 69 **Tm** 168.93	ytterbium 70 **Yb** 173.04
**Actinide series	actinium 89 **Ac** [227]	thorium 90 **Th** 232.04	protactinium 91 **Pa** 231.04	uranium 92 **U** 238.03	neptunium 93 **Np** [237]	plutonium 94 **Pu** [244]	americium 95 **Am** [243]	curium 96 **Cm** [247]	berkelium 97 **Bk** [247]	californium 98 **Cf** [251]	einsteinium 99 **Es** [252]	fermium 100 **Fm** [257]	mendelevium 101 **Md** [258]	nobelium 102 **No** [259]

Karlsruher` chart of nuclides

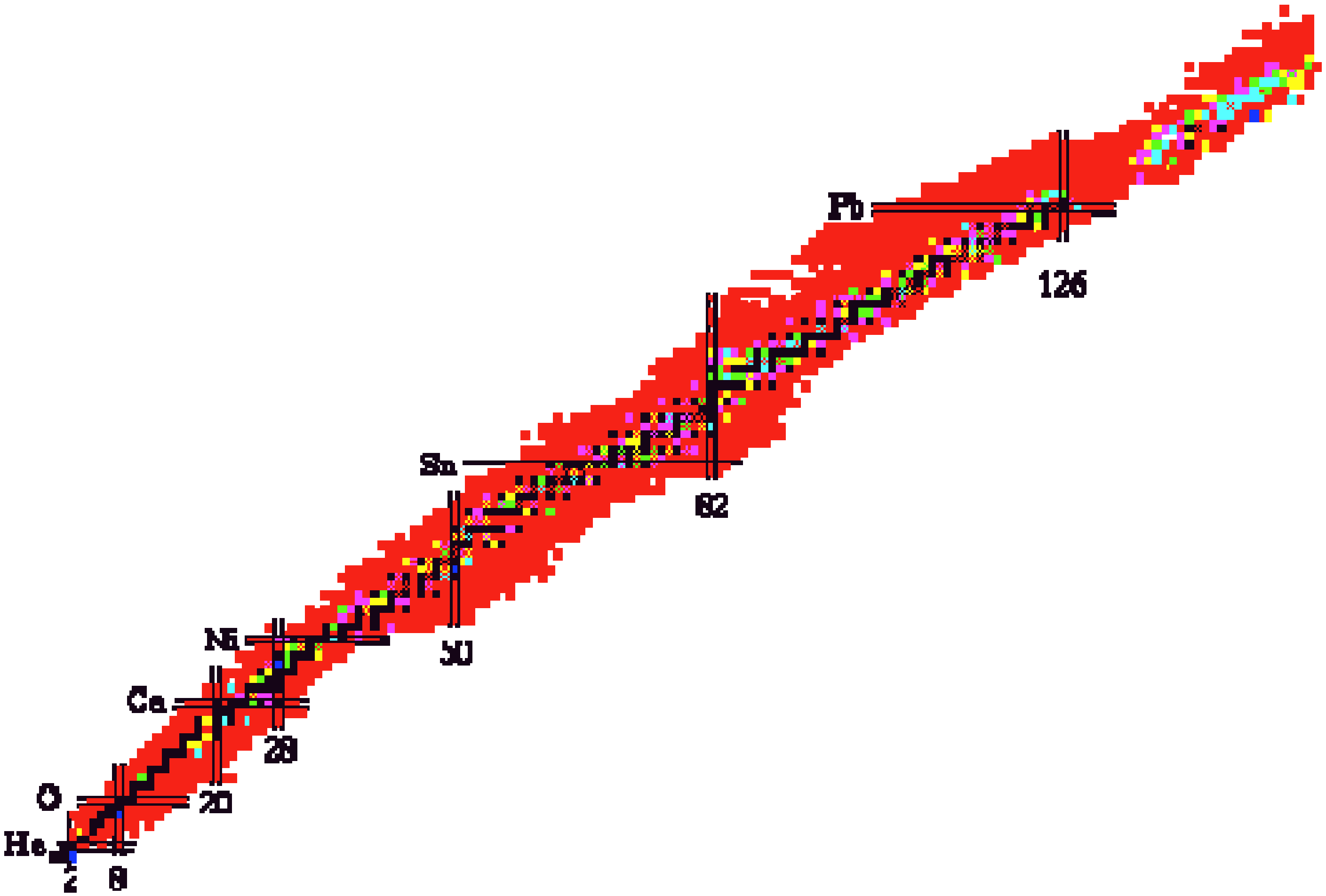
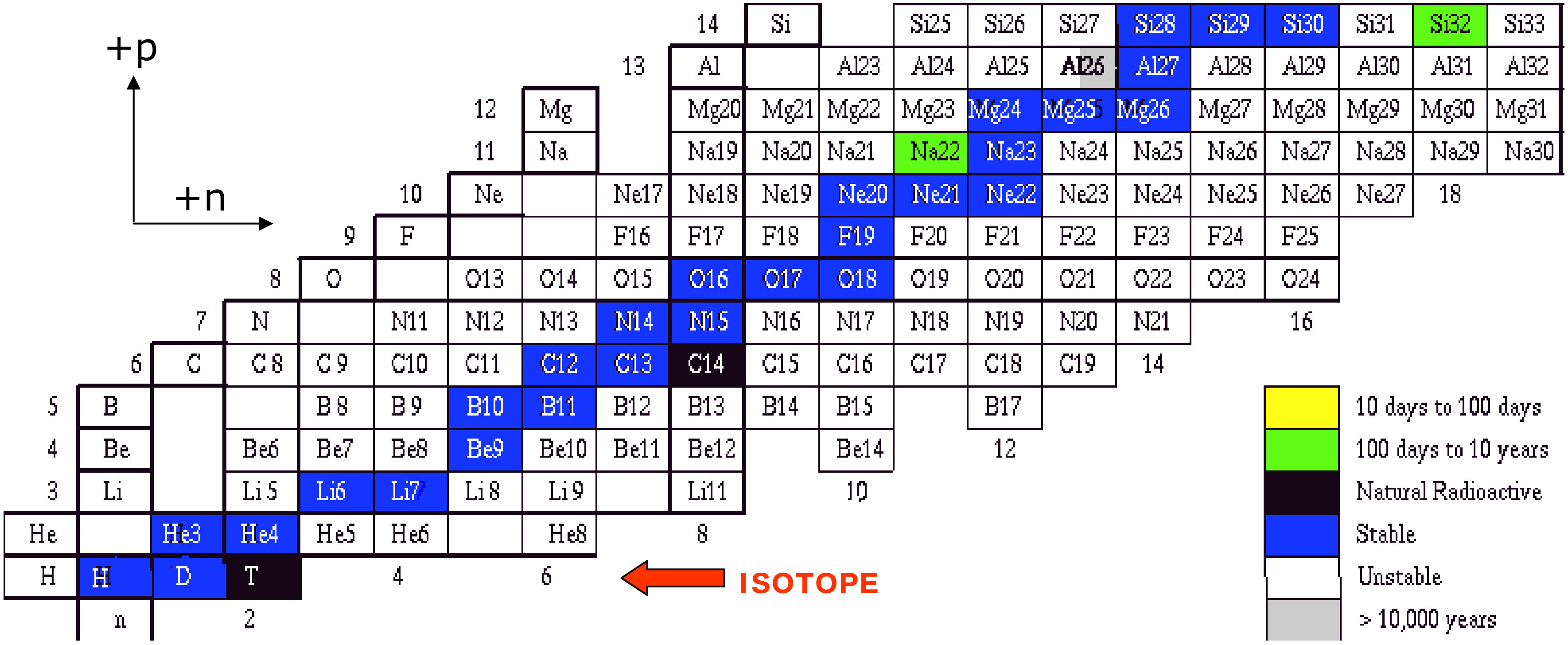


Chart of nuclides, lower section



									14	Si		Si25	Si26	Si27	Si28	Si29	Si30	Si31	Si32	Si33
								13	Al		Al23	Al24	Al25	Al26	Al27	Al28	Al29	Al30	Al31	Al32
						12	Mg		Mg20	Mg21	Mg22	Mg23	Mg24	Mg25	Mg26	Mg27	Mg28	Mg29	Mg30	Mg31
						11	Na		Na19	Na20	Na21	Na22	Na23	Na24	Na25	Na26	Na27	Na28	Na29	Na30
					10	Ne		Ne17	Ne18	Ne19	Ne20	Ne21	Ne22	Ne23	Ne24	Ne25	Ne26	Ne27	18	
				9	F			F16	F17	F18	F19	F20	F21	F22	F23	F24	F25			
			8	O		O13	O14	O15	O16	O17	O18	O19	O20	O21	O22	O23	O24			
		7	N		N11	N12	N13	N14	N15	N16	N17	N18	N19	N20	N21		16			
	6	C	C 8	C 9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	14					
5	B			B 8	B 9	B10	B11	B12	B13	B14	B15		B17							
4	Be		Be6	Be7	Be8	Be9	Be10	Be11	Be12		Be14		12							
3	Li		Li 5	Li6	Li7	Li 8	Li 9		Li11		10									
He		He3	He4	He5	He6		He8		8											
H	H	D	T		4		6													
	n		2																	

🟨 10 days to 100 days
🟩 100 days to 10 years
⬛ Natural Radioactive
🟦 Stable
⬜ Unstable
▨ > 10,000 years

Stable isotopes and radioisotopes

- ⇨ **Half-lifes** of „stable" isotopes are >10^9 years
- ⇨ The stability of atomic nuclei depends on the **ratio p : n**.
- ⇨ Unstable isotopes undergo **nuclear transformations** to form more stable daughter nuclei (isotopes) and thereby emit characteristic nuclear radiation

Stable isotopes in ecology

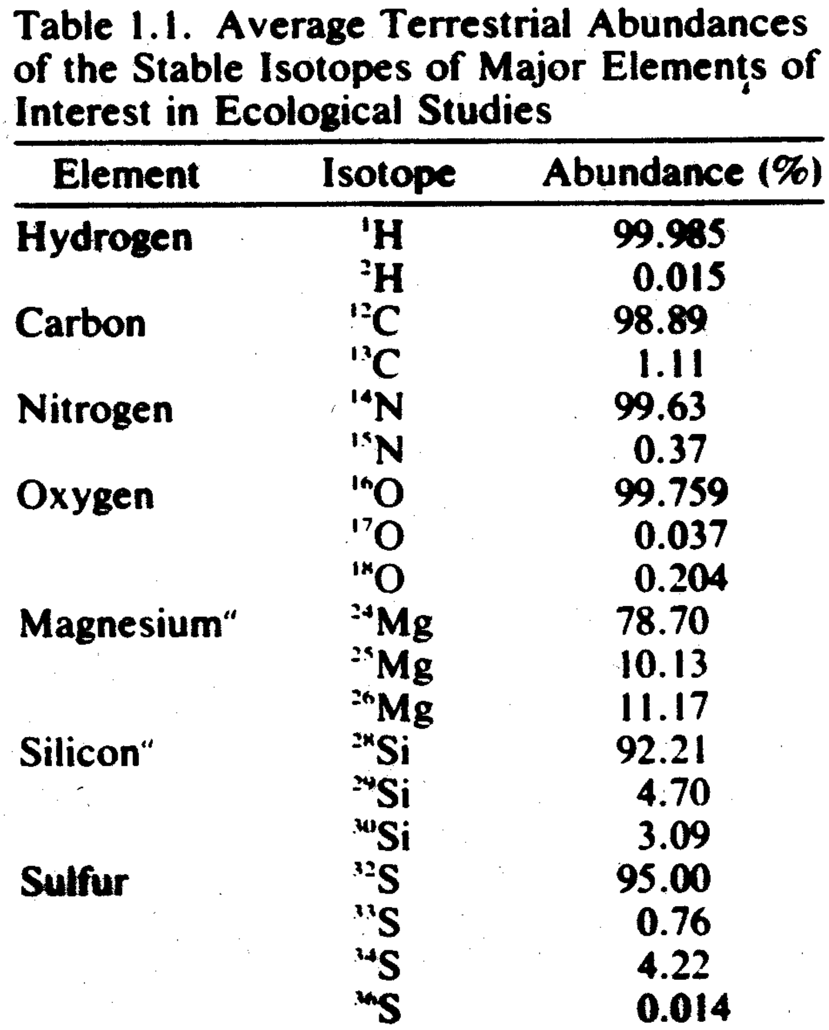


Table 1.1. Average Terrestrial Abundances of the Stable Isotopes of Major Elements of Interest in Ecological Studies

Element	Isotope	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Magnesium[a]	^{24}Mg	78.70
	^{25}Mg	10.13
	^{26}Mg	11.17
Silicon[a]	^{28}Si	92.21
	^{29}Si	4.70
	^{30}Si	3.09
Sulfur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{36}S	0.014

Natural isotope abundances

Isotopic composition is expressed relative to a standard

$$\delta\ [‰] = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

Example: $$\delta^{15}N\ [‰] = \left(\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{std}} - 1\right) \times 1000$$

δ notation is unitless (‰ only indicates multiplication)

Isotope Ratio

$$R = \frac{\text{(amount of heavy isotope)}}{\text{(amount of light isotopes)}}$$

Example: $R^{15}N = (^{15}N\ /\ ^{14}N)$

Delta notation

Natural isotope abundances are given in delta notation which expresses the relative deviation of the isotope ratio of a sample from that of a international standard (‰)

Isotope abundances in tracer studies

$$\textbf{Atom \%} = \frac{\text{(amount of heavy isotope)}}{\text{(amount of all isotopes)}}$$

Example: $\text{atom\% } {}^{15}N = \frac{{}^{15}N}{({}^{14}N + {}^{15}N)} \times 100$

APE = atom percent excess

$\text{APE} = \text{at\%}_{\text{sample}} - \text{at\%}_{\text{control}}$

(controls: natural abundance or non-treated samples)

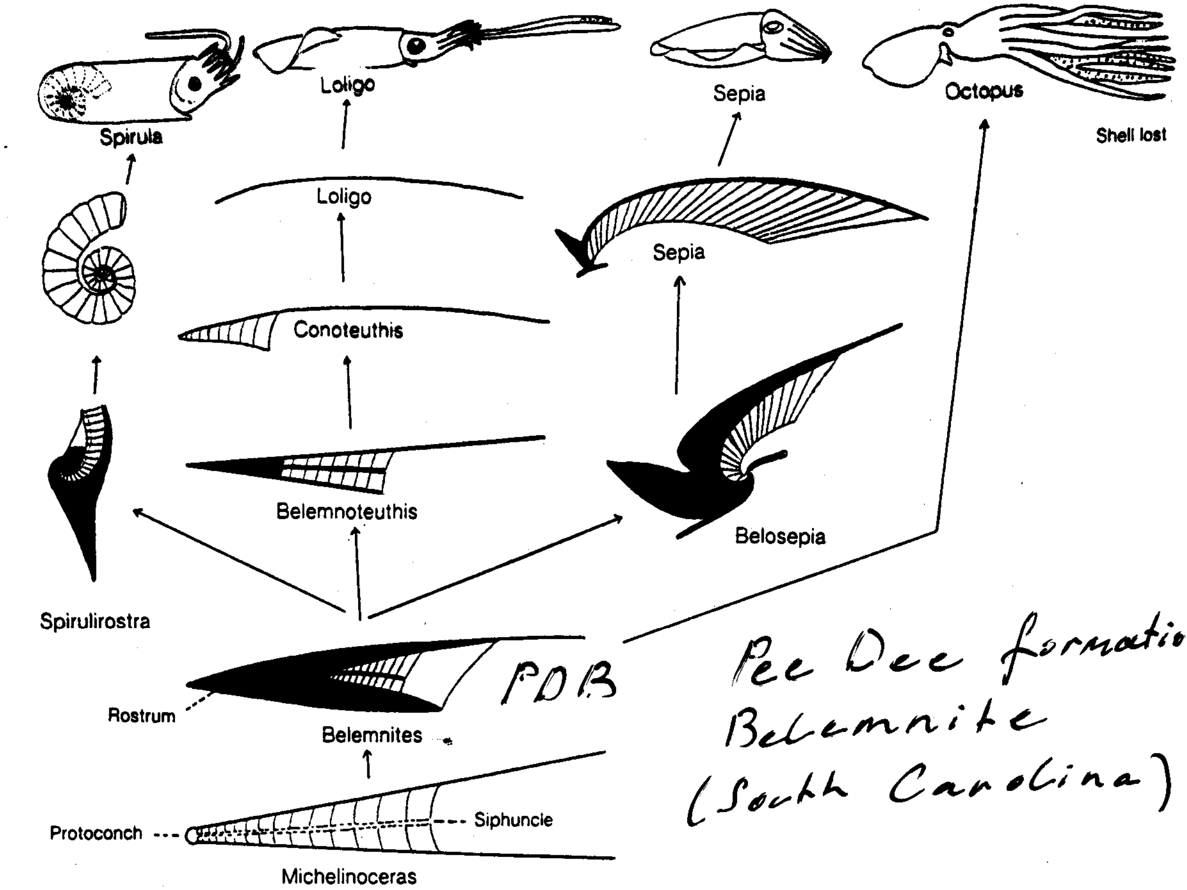
Isotopes of light elements

Elem.	Isotope	Delta	Reference material
H	^{1}H, ^{2}H (D)	δD	SMOW
C	^{12}C, ^{13}C	$\delta^{13}C$	V-PDB
N	^{14}N, ^{15}N	$\delta^{15}N$	at-air
O	^{16}O, ^{17}O, ^{18}O	$\delta^{18}O$	SMOW
S	^{32}S, ^{33}S, ^{34}S, ^{36}S	$\delta^{34}S$	CD

SMOW	Standard Mean Ocean Water
V-PDB	Vienna Pee Dee Belemnite
at-air	N_2 in atmospheric air
CDT	Canon Diablo Meteorite

International reference materials

z.B. Vienna Pee Dee Belemnite



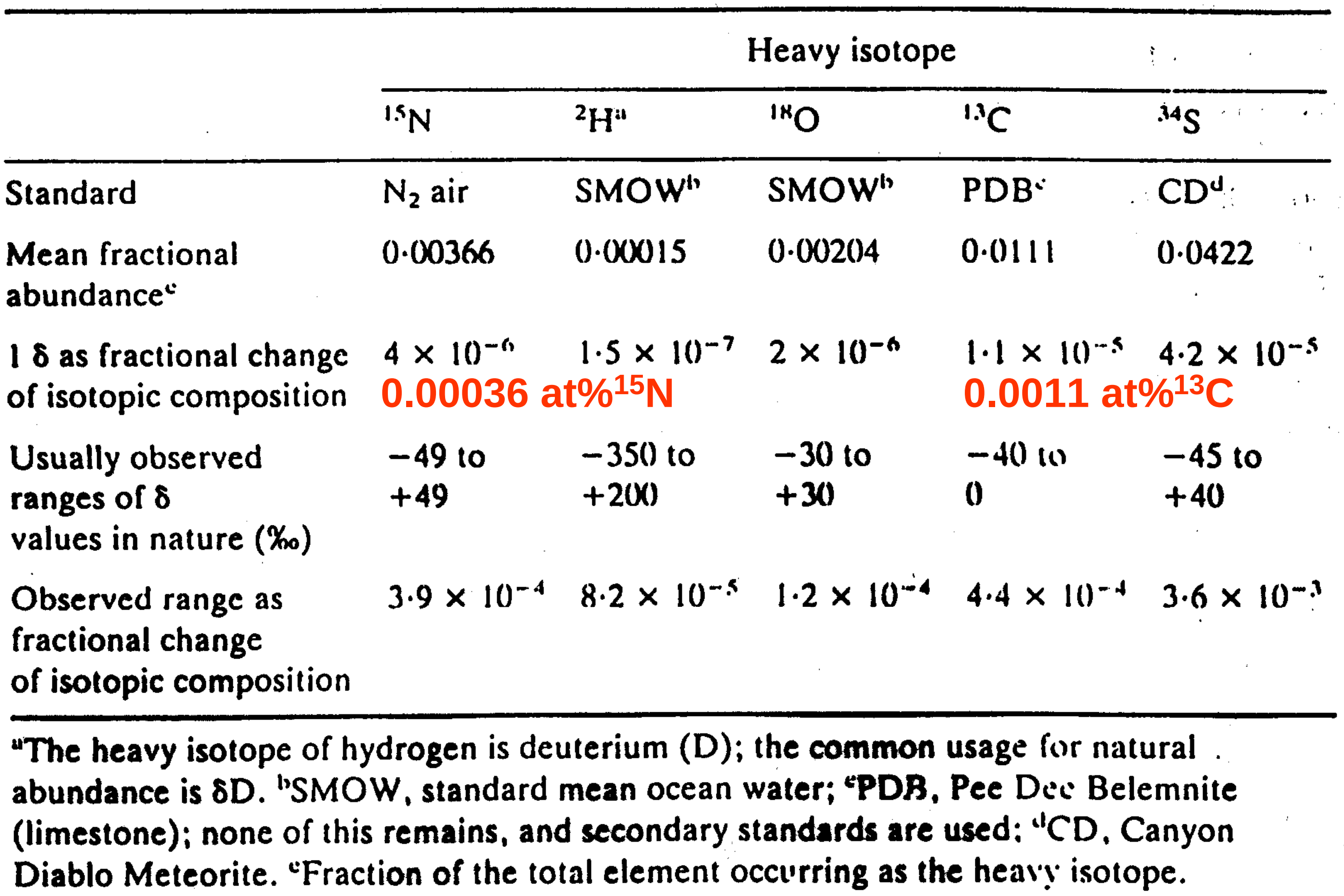
PDB

Pee Dee formation
Belemnite
(South Carolina)

Figure 11–97 Evolution of shells of the Coleoidea. (After Shrock and Twenhofel.)

Certified reference materials are available from IAEA (International Atomic Energy Agency) or NIST (National Institute of Standards)

Abundances of isotopes



	Heavy isotope				
	^{15}N	^{2}H[a]	^{18}O	^{13}C	^{34}S
Standard	N_2 air	SMOW[b]	SMOW[b]	PDB[c]	CD[d]
Mean fractional abundance[e]	0·00366	0·00015	0·00204	0·0111	0·0422
1 δ as fractional change of isotopic composition	4×10^{-6} 0.00036 at%^{15}N	$1{\cdot}5 \times 10^{-7}$	2×10^{-6}	$1{\cdot}1 \times 10^{-5}$ 0.0011 at%^{13}C	$4{\cdot}2 \times 10^{-5}$
Usually observed ranges of δ values in nature (‰)	−49 to +49	−350 to +200	−30 to +30	−40 to 0	−45 to +40
Observed range as fractional change of isotopic composition	$3{\cdot}9 \times 10^{-4}$	$8{\cdot}2 \times 10^{-5}$	$1{\cdot}2 \times 10^{-4}$	$4{\cdot}4 \times 10^{-4}$	$3{\cdot}6 \times 10^{-3}$

[a] The heavy isotope of hydrogen is deuterium (D); the common usage for natural abundance is δD. [b] SMOW, standard mean ocean water; [c] PDB, Pee Dee Belemnite (limestone); none of this remains, and secondary standards are used; [d] CD, Canyon Diablo Meteorite. [e] Fraction of the total element occurring as the heavy isotope.

Comparison of notations

	R ^{15}N	atom% ^{15}N	$\delta^{15}N$ [‰]
Plant	0.003680	0.36662	1.0
Soil	0.003685	0.36717	2.5
Herbivore	0.003691	0.36779	4.2

Isotopic differences in natural material

depleted
("light")

enriched
("heavy")

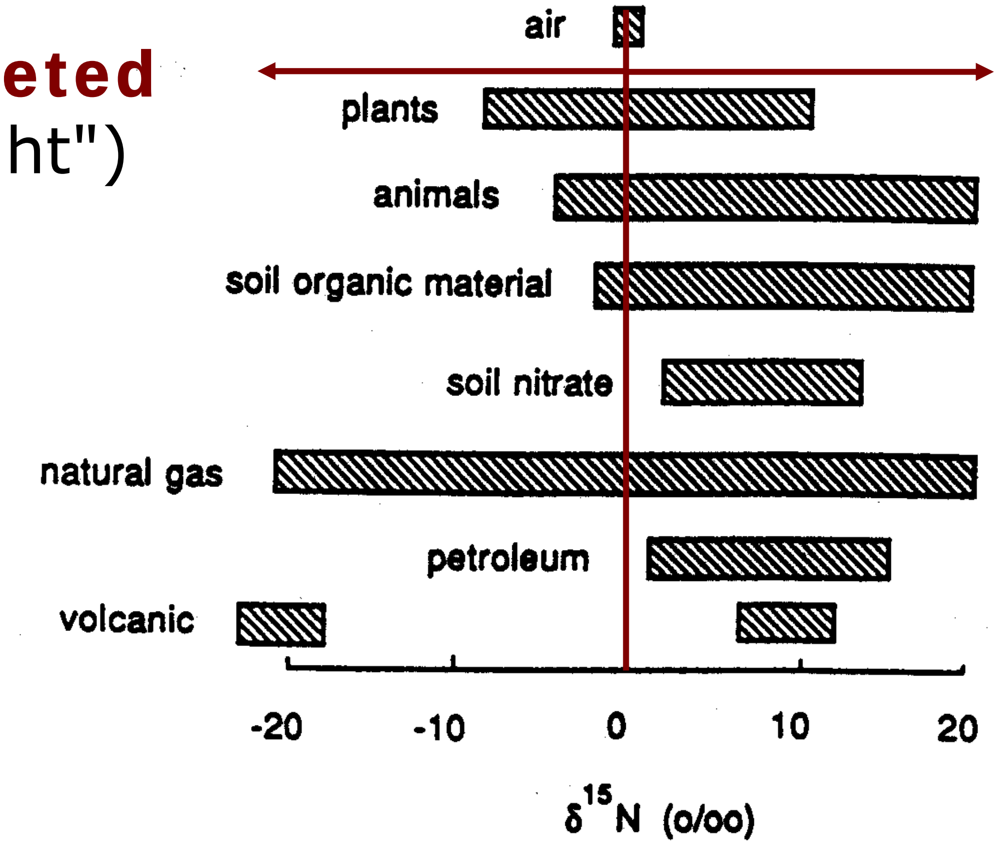
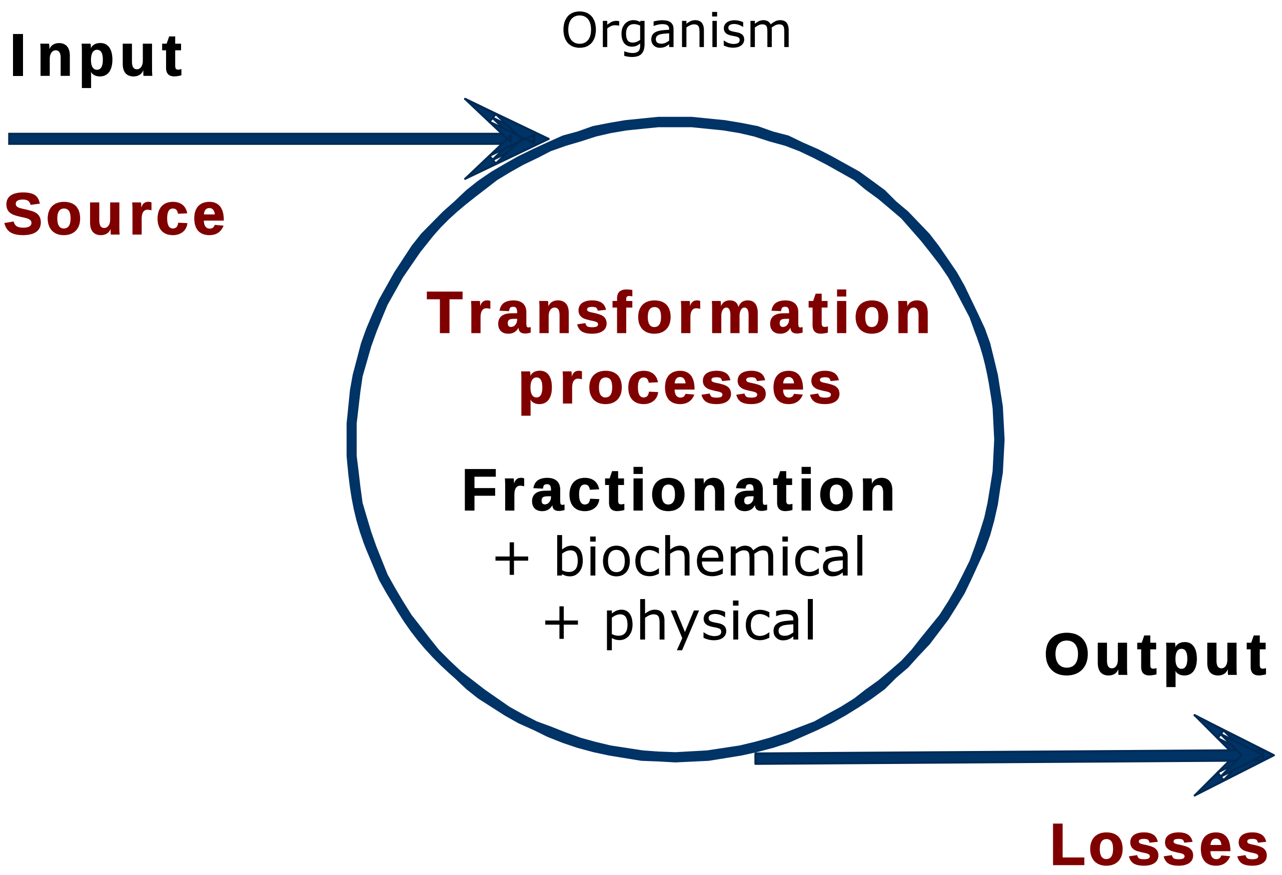


Figure 1.6. Observed ranges of nitrogen isotope ratios from various substances.

Controls of isotopic composition



Input

Source

Organism

Transformation processes

Fractionation
+ biochemical
+ physical

Output

Losses

Isotope fractionation

Isotope fractionation may occur in a reaction sequence or between a diet and an organism.

Isotope fractionation in life sciences is commonly expressed as **isotope discrimination Δ** (large delta, equivalent to enrichment factor ε)

$$\Delta = \delta_{Substrate} - \delta_{Product} = 1000 * (\alpha - 1), \text{ given in ‰}$$

Discrimination is related to the **fractionation factor α**.
$\alpha_{S/P} = R_A / R_B$; or $\alpha = \Delta / 1000 + 1$

Example: $\alpha = 1.002 \sim \Delta = 2‰$
product isotopically depleted in heavier isotope by 2‰ compared to the substrate

Potential energy differences in bonds

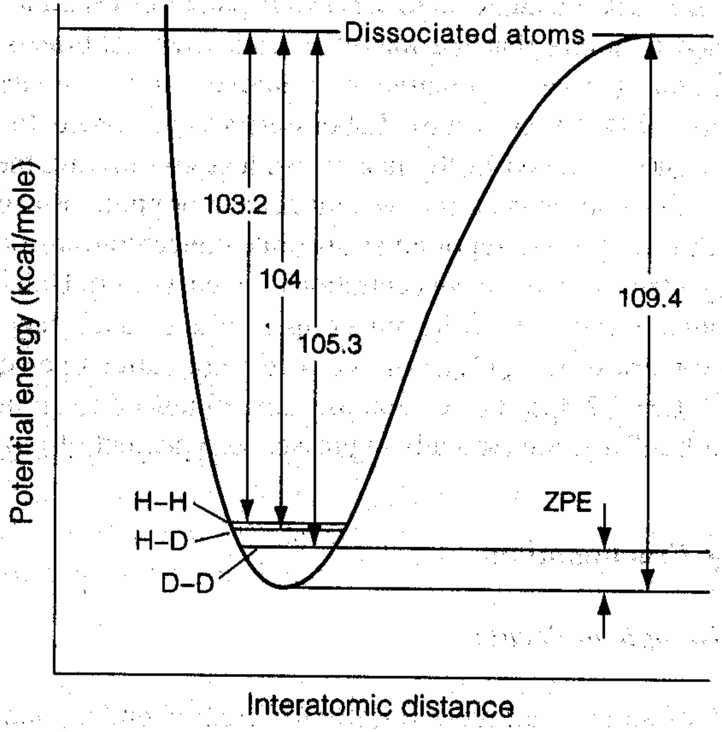


Figure 2.2. The interatomic distance - potential energy relationship for stable hydrogen isotopes of a molecule. Higher zero point energies (ZPE) result in molecules being less stable. Modified from O'Neil (1986).

Isotopically heavier atoms are more strongly bonded and have lower zero point energies (ZPE) than lighter atoms, due to lower vibrational energy → to break such a bond more energy is required

Kinetic isotope effects

substrate A ⟶ product B

$${}^{light}A \overset{{}^{light}k}{\Rightarrow} {}^{light}B$$

$${}^{heavy}A \overset{{}^{heavy}k}{\Rightarrow} {}^{heavy}B$$

„irreversible“, chemical of physical transition between A and B, unidirectional

$\alpha_{\mathbf{kin}} = {}^{\mathbf{light}}\mathbf{k} \,/\, {}^{\mathbf{heavy}}\mathbf{k}$, most frequently > 1.0

Isotopically heavier molecules react slower than isotopically lighter molecules. KIEs are not additive. Higher **binding energies** of molecules of greater mass, More energy necessary to break bonds of isotopically heavier molecules.

Kinetic isotope effects

$$\alpha_{kin} = {}^{light}k \; / \; {}^{heavy}k$$

Diffusion, evaporation and **enzymatic reactions** frequently discriminate against the isotopically heavier substrate/isotope.

Isotopic depletion of the product by

Nitrate reductase	Δ = 15‰ (α = 1.015)
Rubisco	Δ = 28‰
Ammonium oxidation	Δ = 60‰
PEP carboxylase	Δ = 2‰
CO_2 diffusion	Δ = 4‰

Fractionation in a closed system

<u>Raleigh fractionation</u>

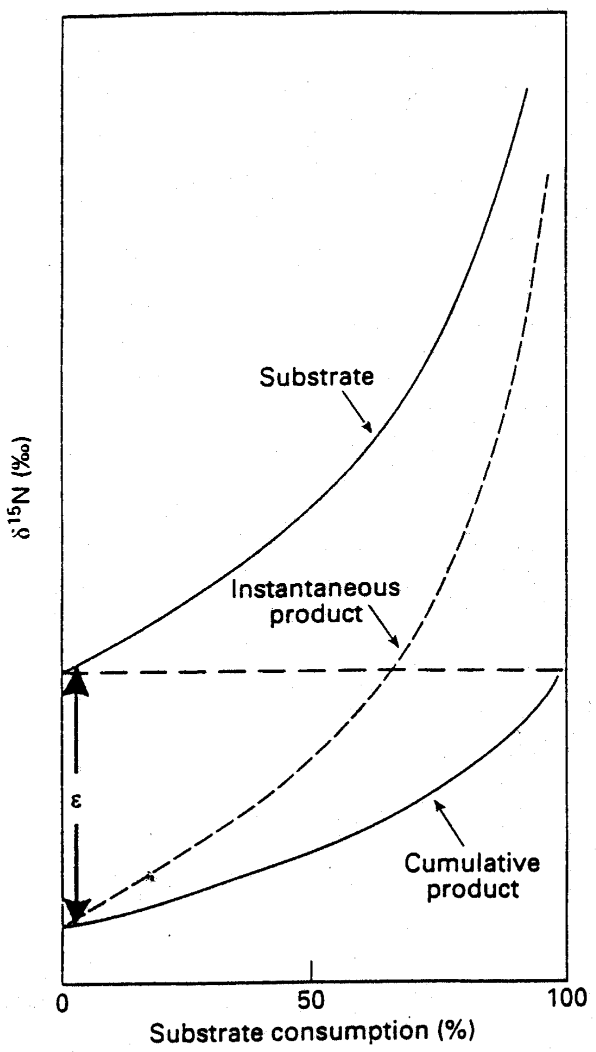
- fractionation $\alpha > 1$
  product depleted

- substrate becomes increasingly isotopically enriched

  cumulative product approaches isotopic composition of initial substrate

- if all substrate is consumed:
  δ product = δ original substrate
  no apparent discrimination!!

- Only in semi-closed or closed systems i.e. dependent on substrate supply:demand or substrate pool size



Equilibrium isotope effects

$$\textbf{substrate} \underset{\alpha_{\mathbf{back}}}{\overset{\alpha_{\mathbf{forward}}}{\rightleftharpoons}} \textbf{product}$$

$$\alpha_{equ} = \alpha_{forw}/\alpha_{back}$$

$$[\alpha_{forw} = {}^{light}k_{forw} \,/\, {}^{heavy}k_{forw}]$$

Integral of the kinetic IEs of back and forward reaction.

Occur during isotope exchange reactions.

Finally isotopes are **unequally distributed** in isotopic equilibrium between two phases, states of aggregation or molecules.

Equilibrium isotope effects

Higher **activation energy** needed to dissociate isotopically heavier molecules. Additive. Temperature-dependent: zero potential energies diminish and therefore equilibrium isotope effects decrease

Rules of thumb - enrichment of the heavier isotope in

- compounds of higher **oxidation state** e.g. sulphate > sulfide
- compounds of greater **density** of aggregation state
  e.g. snow > water > moisture
- compounds of greater **molecular mass**
  e.g. carbonate > bicarbonate > CO_2

e.g. ^{15}N *enrichment of* NH_4^+ *relative to* NH_3 (Δ = -25 ‰), NH_3 *hydratation*
^{13}C *enrichment of* HCO_3^- *relative to* CO_2 (Δ = -8 ‰), CO_2 *Hydratation*

Mass differences in molecules

e.g. Glucose

$^{12}C_6{}^1H_{12}{}^{16}O_6$ (180) $\Rightarrow$ $^{13}C^{12}C_5{}^1H_{12}{}^{16}O_6$ (181)

Mass difference 1/181

Glucose is not volatile. Derivatisation – extra-C!

e.g. Carbon dioxide

$^{12}C^{16}O_2$ (44) $\Rightarrow$ $^{13}C^{16}O_2$ (45)

Mass difference 1/45

Therefore: sample preparation –
combustion of samples to simple gases before isotope ratios are measured

Isotope analysis

Gas isotope analysis by gas isotope ratio mass spectrometry (IRMS)

+ Small molecular weight gases to detect differences in isotopic abundance and mass
+ Ionisation and separation of gases by mass:charge ratio
+ No direct absolute measurement of isotopic abundances but against reference materials/ gases to detect the small differences in isotopic abundances

Dual-Inlet IRMS

- Off-line preparation of gases (C to CO_2, N to N_2)
- Repeated measurements of sample versus standard gas
- Highest precision
- Low sample throughput due to laborious off-line sample preparation (<10/day)

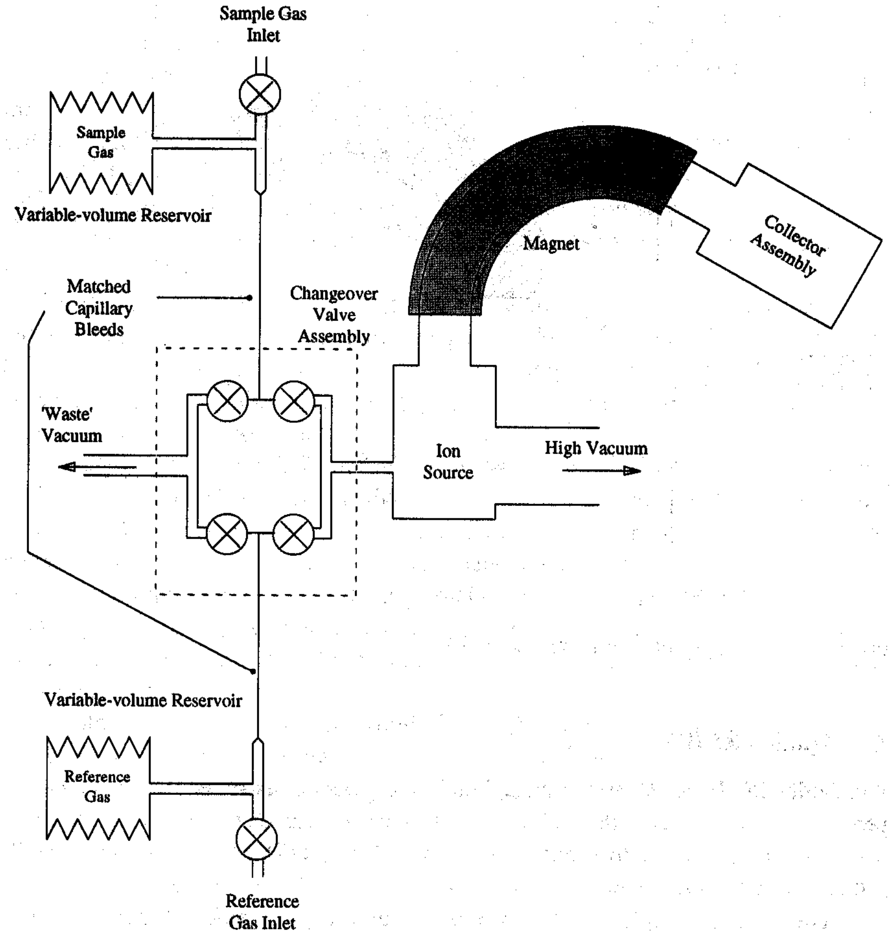
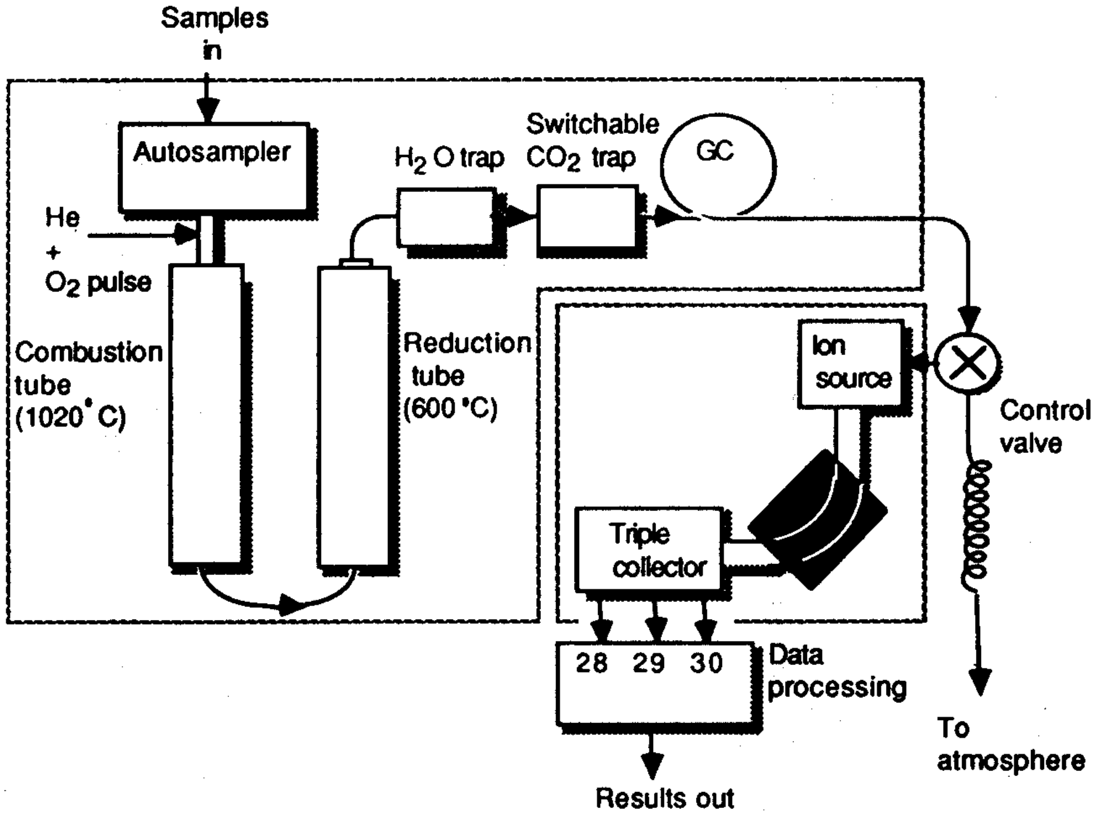


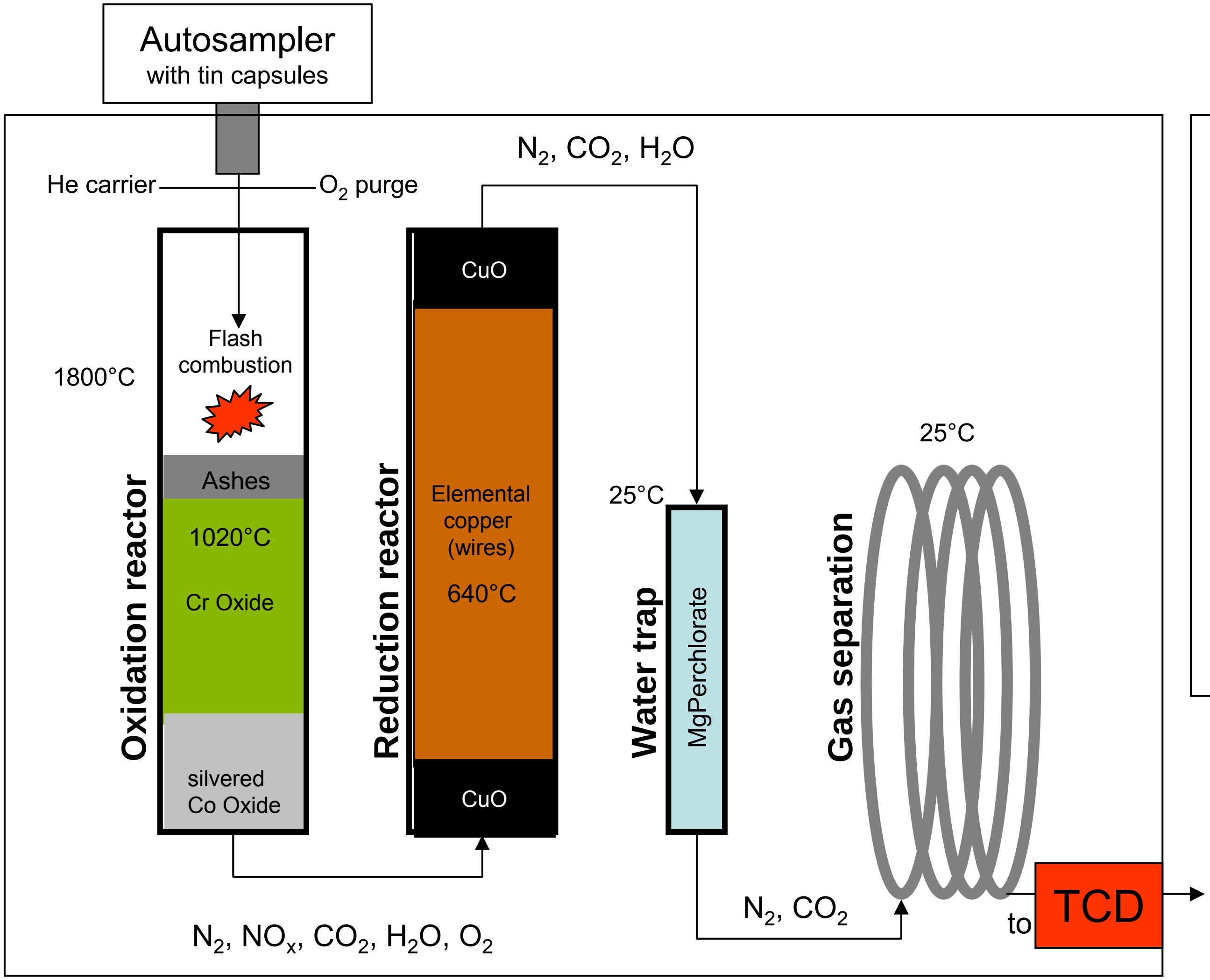
Figure 5 Principles of dual-inlet IRMS.

Continuous-flow IRMS

- E.g. coupling of an elemental analyzer to IRMS via interface
- High temperature conversion of (in)organic matter to CO_2 and N_2
- He as carrier gas
- High throughput (>100/day)
- Lower precision



EA - principle

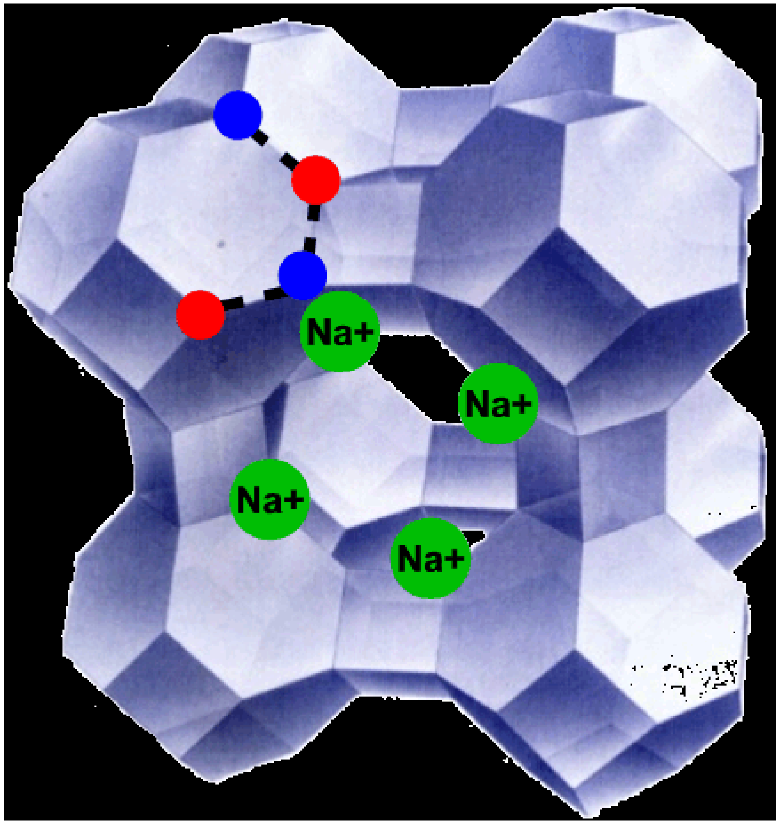


Function

- Combustion of solid/ liquid samples
- Trapping of H_2O and SO_2
- Separation of N_2 and CO_2
- Quantification by thermal conductivity detector

Gas separation

- Gas separation by molecular sieves i.e. crystalline metal aluminosilicates having a three-dimensional interconnecting network of silica and alumina tetrahedra. Uniform cavities selectively adsorb molecules of a specific size.
- 4- to 8-Å sieves are normally used in gas phase applications.



Molecular sieve 4Å

Interface EA – IRMS

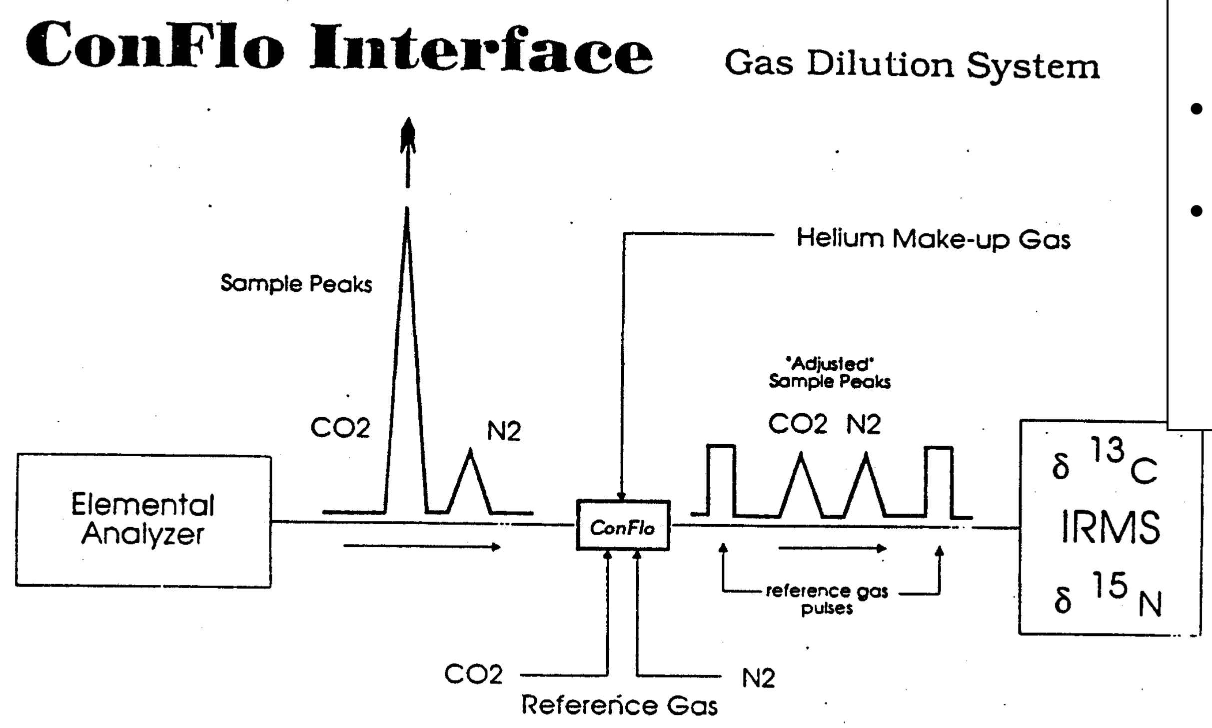
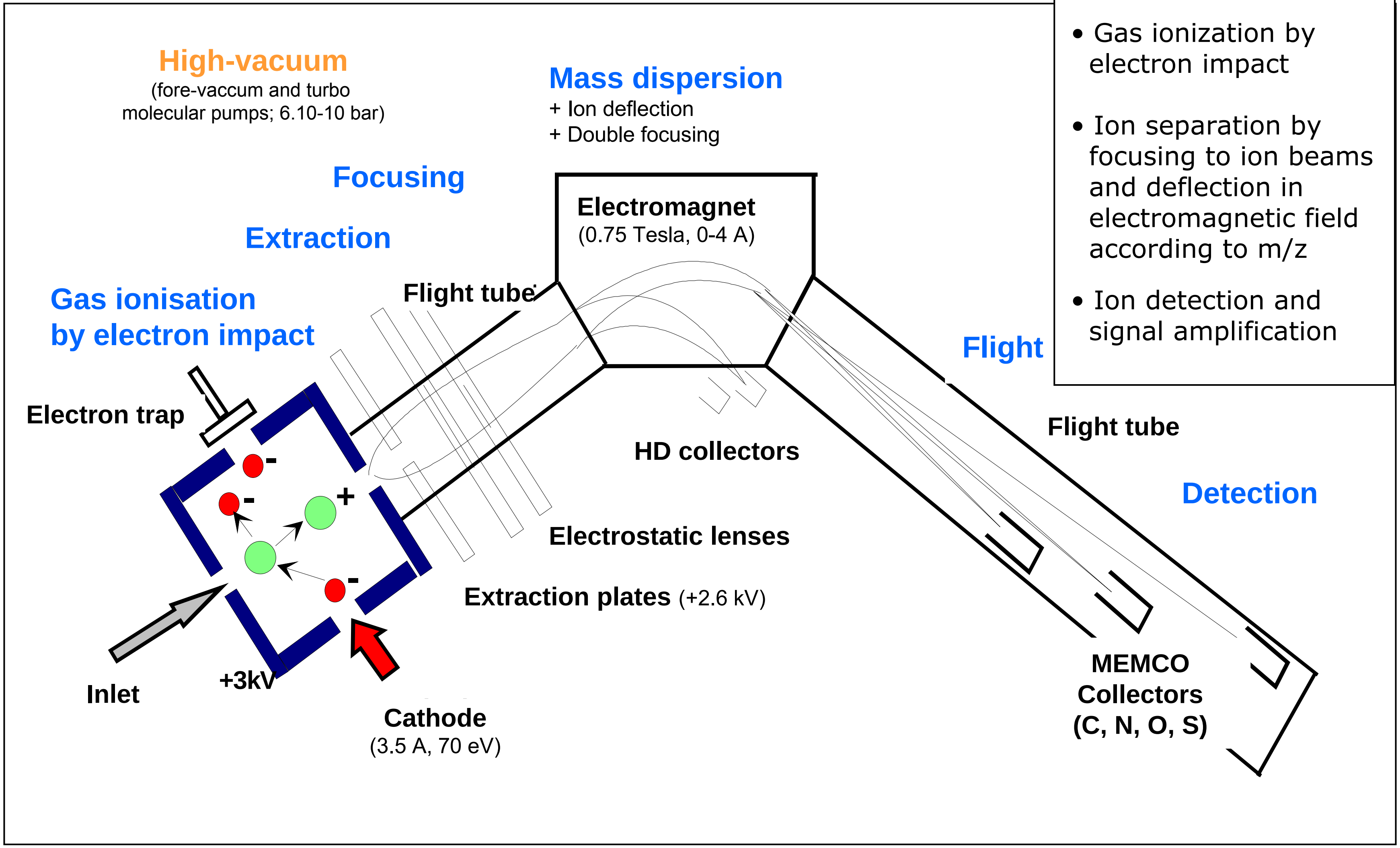


Fig.2a Flow chart ConFlo

Function

- Reference gas injection
- Adjustment of N_2/CO_2 signals by He dilution
- Decrease of He flow by open split to maintain constant high-vacuum in ion source

IRMS



Function

- Gas ionization by electron impact
- Ion separation by focusing to ion beams and deflection in electromagnetic field according to m/z
- Ion detection and signal amplification

Gas ionisation by electron impact

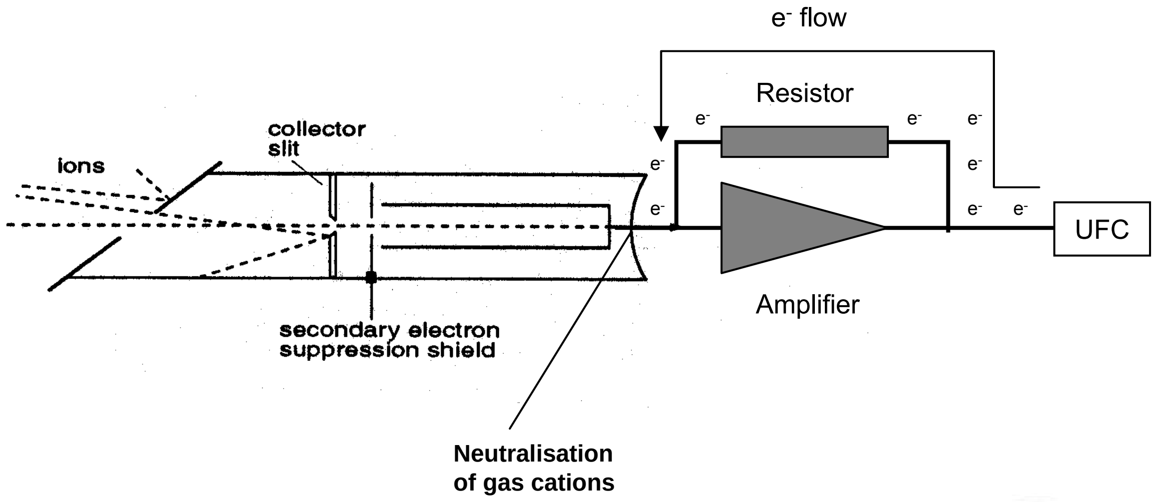
N_2	--->	N_2^+	m/z	28
				29
				30
		N^+	m/z	14
CO_2	--->	CO_2^+	m/z	44
				45
				46
		O_2^+	m/z	32
		CO^+	m/z	28
		C^+	m/z	12

CO_2^+ (45) $^{12}C^{17}O^{16}O$, $^{13}C^{16}O^{16}O$
CO_2^+ (46) $^{12}C^{18}O^{16}O$, $^{12}C^{17}O^{17}O$, $^{13}C^{16}O^{17}O$ etc.
Correction for oxygen isotope contribution necessary

Ion detection

Multielement Multicollector – MEMCO

Measurement of ion flux through neutralisation of gas cations in the detector; cup 1-3 (m/z 28-30), 4-6 (m/z 44-46), 3/4 (m/z 62/64)

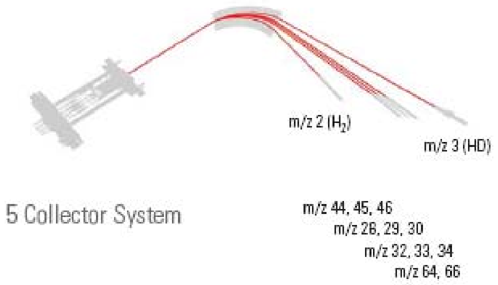


Universal triple collector

for CO_2 and N_2O (44-46), N_2 (28-30) and CO (28-30)

two extra collectors for H_2 (2, 3)

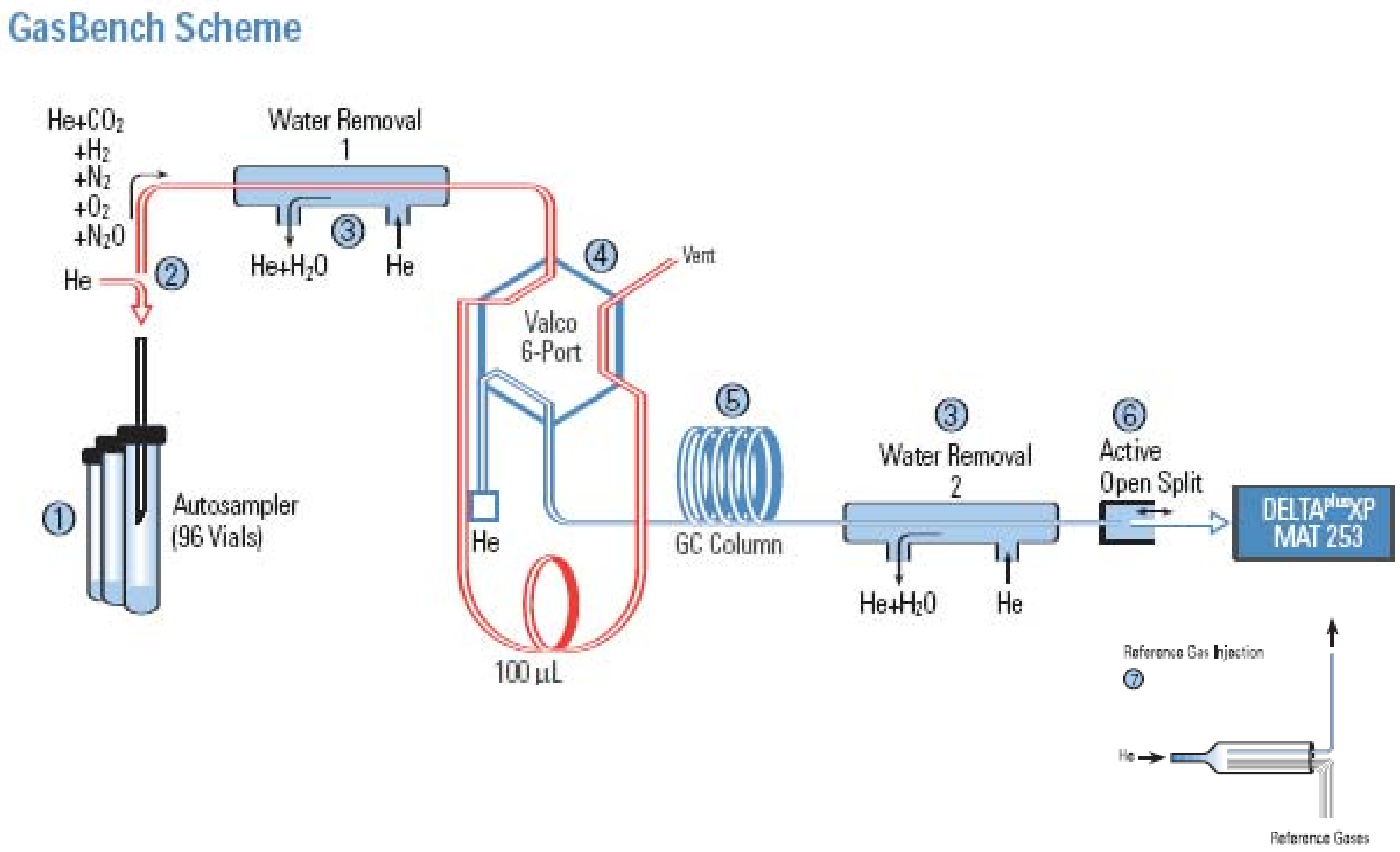
Helium suppression in cup 3



Trace gas analysis - Gas Bench II

- On-line isotope analysis in headspace samples, e.g. water equilibration, carbonates, atmospheric gases (CO_2, CH_4, N_2O, N_2, H_2)
- Multiple loop injection for enhanced precision
- GC separation of gas mixtures
- Cryogenic focusing of trace gases from large air volumes prior to GC separation

Gas Bench II – CO_2 measurement

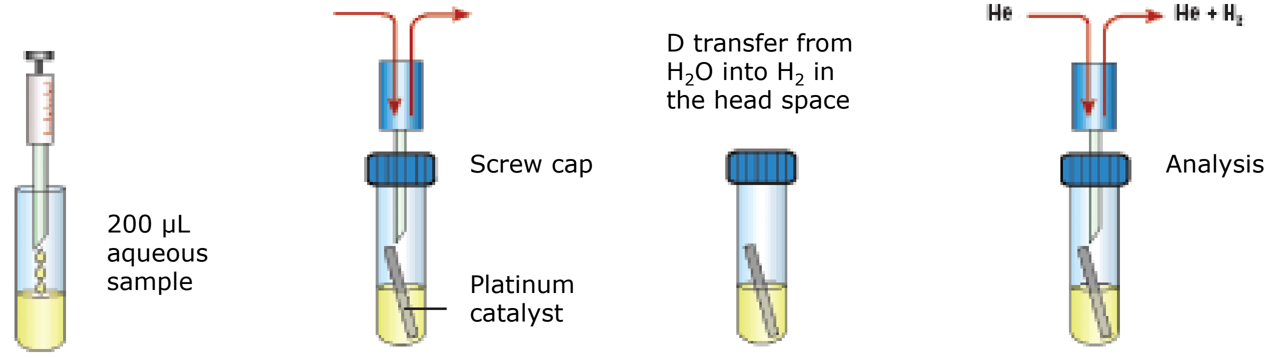


GasBench Scheme

Gas Bench II – Water equilibration

- $H_2O:CO_2$ equilibration for $\delta^{18}O$ $$H_2{}^{18}O + CO_2 \leftrightarrow H_2O + C^{18}O_2$$
- $H_2O:H_2$ equilibration for $\delta^{2}H$ (δD): $${}^{2}H_2O + H_2 \leftrightarrow H_2O + {}^{2}H_2$$

Pt catalyst



- Place 200 µL of the sample in the vial

- Place all sample vials in the autosampler tray
- All vials are automatically flushed

- Eqilibrate for 40 min

- Start the sequence acquisition

Compound-specific isotope analysis

Compound-specific Isotope Analysis (CSIA)

LC-IRMS (Liquid chromatography)
$\delta^{13}C$ in sugars, organic & amino acids etc.

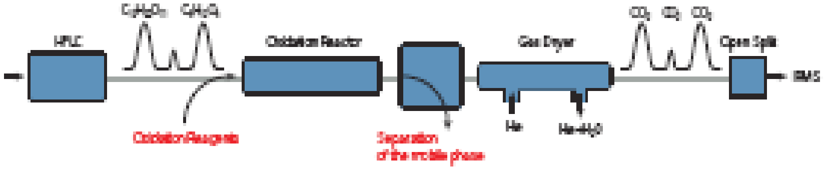


Figure 2-1. Analysis Principle of Finnigan LC IsoLink

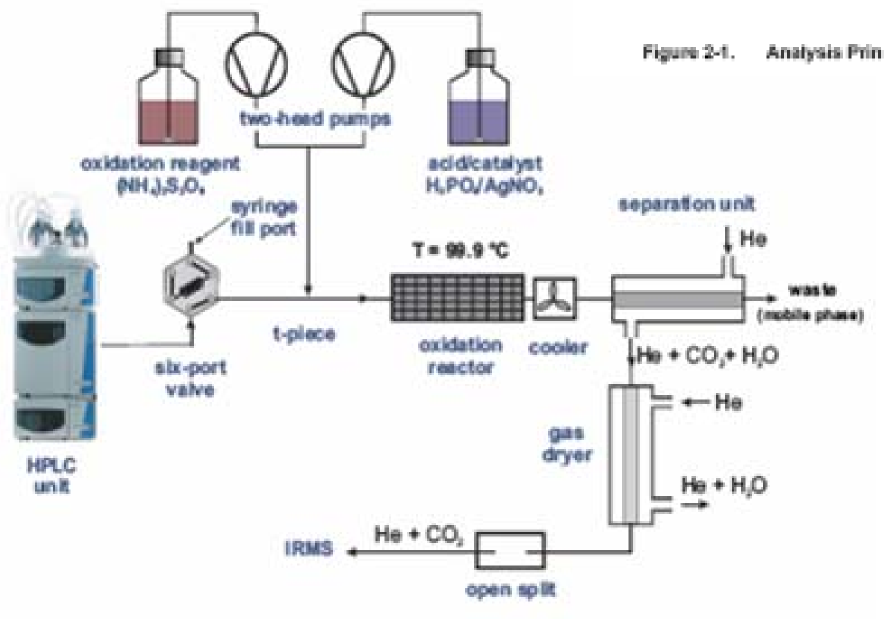


Figure 2-5. Scheme of Finnigan LC IsoLink interface and Finnigan Surveyor® HPLC Unit