What is chromatography?

Chromatography, together with the various forms of spectroscopy and spectrometry (see Box D, Spectroscopy and spectrometry), represent the two major basic analytical techniques, the former serving for the separation, the latter for the identification of the constituents of a substance.

Chromatography (from the Greek khrôma, "color," and graphein, "to write"), allows the separation of the constituents of a mixture in a homogeneous liquid or gaseous phase, as blotting paper might spread out in concentric rings a liquid poured onto

A chromatograph comprises a sample injection device, a column, a detector, and a recording and analysis system. Its principle is based on the equilibrium of compound concentrations, between two phases coming into contact: the stationary phase, in the column, and the *mobile phase*, which moves across it. Separation relies on the differential displacement of constituents inside the column, passing through in times that are proportional to their size, or depending on their structure, or affinity for the stationary phase (polarity...). As they reach the far end of the column, a detector measures, on a continuous basis, the quantities of each constituent.

The most common form of chromatography is qas chromatography, carried out on gaseous samples, or samples that may be vaporized without incurring breakdown. The mobile phase is a gas (helium, nitrogen, argon, or hydrogen), constantly sweeping through the column, which is placed in a thermostat oven. Detectors allow the selective analysis and identification of highly complex mixtures.

If the stationary phase is a nonvolatile, or not highly volatile liquid, exhibiting solvent properties for the compounds to be separated, the process is termed gas-liquid chromatography, or partition chroma-

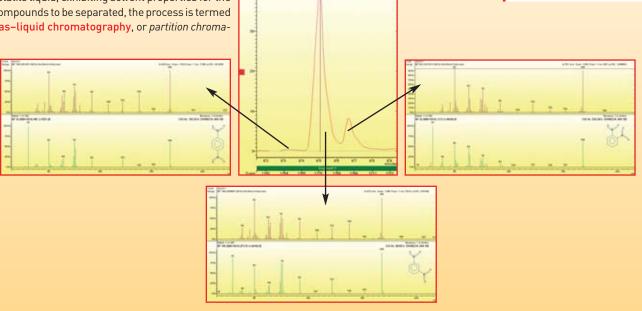
tography. If the stationary phase is an adsorbent solid (silica, alumina, zeolites, or polymers), this is gas-solid chromatography. Within this same family, of adsorption chromatography processes, liquid-solid chromatography is characterized by its stationary phase, this being a polar solid.

In high-performance liquid chromatography (HPLC), the sample must be wholly soluble in the mobile phase (elution solvent). The latter must be kept at high pressure (hence the alternative name of high-pressure liquid chromatography), to ensure a constant flow rate inside the column, and preclude any loss of head. HPLC involves solute-mobile phase-stationary phase exchange mechanisms, based on partition or adsorption coefficients, depending on the nature of the phases in contact.[1]

A chromatographic analysis yields a chromatogram, this being a graphical representation of the evolution of a parameter (intensity of the detector signal), related to instantaneous solute concentration, as function of time. This exhibits peaks, rising above the baseline, which obtains in the absence of any compounds (see Figure).

(1) There are two further types of liquid chromatography, ion chromatography, and exclusion chromatography.

N.B: This Box reproduces a number of excerpts from a presentation by Pascale Richardin, head of the Datation Group at the Research and Restoration Center of the French National Museums Administration (Musées de France), taken from the pages dealing with analytical methods, as posted on the site: ttp://www.culture.gouv.fr/culture/conservation/fr/ biblioth/biblioth.htm



An example of the combined use of mass spectrometry and chromatography: the separation of isomers ("sister molecules") of an explosive molecule (dinitrobenzene [DNB]), after solid-phase microextraction sampling, by gas chromatography, and their detection by mass spectrometry (SPME-GC-MS).

Spectroscopy and spectrometry

Spectrometric methods are subdivided, as a whole, into two main categories, radiation spectrometry – itself comprising absorption spectrometry, emission spectrometry, Raman scattering spectrometry, and nuclear magnetic resonance spectrometry – and mass spectrometry.

Radiation spectroscopy and spectrometry⁽¹⁾ cover a ensemble of analytical methods allowing the composition and structure of matter to be ascertained, based on investigation of the spectra yielded by the interaction between atoms and molecules, and various types of electromagnetic radiation, emitted, absorbed, or scattered by the former.

Depending on their energy, photons interact selectively with the various electron shells, or levels, making up the electronic structure of the atom, or molecule. The electrons involved are core electrons (close to the atom's nucleus), for X-rays, [2] peripheral electrons (furthest from the nucleus, and involved in chemical bonds) for light absorbed, or emitted, in the near ultraviolet and visible region. In the infrared radiation region, it is the leap from one molecular vibration level to another that is involved the switch from one molecular rotation level to another for microwave radiation, and atomic nucleus spin for NMR.

Absorption spectrometry

Those spectroscopy methods that rely on absorption make use of the Beer-Lambert law, setting out the proportional relation between the intensity of light absorbed, and the amount of absorbing matter:

 $A = \log (I_0/I) = \varepsilon l C,$

where A stands for the absorbance of the medium traversed, I_0 for incident light intensity, I for transmitted light intensity, ϵ is the characteristic molar extinction coefficient, for a given wavelength, for the substance investigated – expressed in

- (1) The term "spectrometry," initially used only to refer to recording and measurement techniques, has tended to become synonymous with "spectroscopy," as the eye was supplanted, for observation purposes, by other receptors and instruments, while the visible region now only formed one special region, in analytical terms.
- (2) It should be noted, at the same time, that X-ray crystallography is not deemed to be a spectroscopy method, in the strict sense of the term

L mol⁻¹ cm⁻¹ – while I stands for the thickness passed through, expressed in centimeters, and C is the concentration, in moles per liter.

By measuring the medium's absorbance, for a given wavelength, the concentration of a substance, in a sample, may thus be determined.

In an absorption spectrum, as recorded by means of a spectrometer, absorption peaks correspond to the wavelengths the medium is able to absorb. Just as the spectrum from the Sun's light is obtained by making it pass through a prism, which breaks it up, spectrometers analyze the spectral distribution of the whole range of electromagnetic radiations, separating them out according to wavelength, by means of a reflection diffraction grating. Spectra exhibit peaks, each one corresponding to a specific wavelength.

Depending of the type of sample to be analyzed, and the performance level being sought, in the laboratory, absorption spectrometry is used either on molecules in liquid or gaseous phase, or on atomic vapor, obtained through thermal breakdown of liquid or solid samples.

Molecular absorption spectroscopy, in the UV-visible region, affords simplicity of use, however it is only applicable to samples of moderate complexity, since, owing to the width of *molecular absorption bands*, absorption spectra, as a rule, do not allow specific discrimination of every constituent, in a complex mixture.

In infrared (IR) spectrometry, absorption is the outcome of molecular vibration and rotation processes. Infrared absorption spectra thus allow the nature of chemical bonds to be determined, that make up a molecule, by ascertaining the bond's elasticity constant (influencing vibration frequency, as for a spring), thus confirming structural hypotheses.

As the number of atoms increases, the spectrum rapidly exhibits growing complexity, and interpretation becomes highly problematical, especially for organic compounds.

Atomic absorption spectrometry, in this respect, brings higher performance, since absorption by atoms yields very narrow absorption lines. Very precise measurements are thus feasible, even when the sample consists in a complex assembly of chemical elements. Atomic absorption is a reference technique for the ana-

lysis of trace elements in a wide variety of samples, in particular for biological samples.

Emission spectrometry

Atoms or molecules brought to an excited state may deexcite by emitting radiation, known as *emission radiation*. When the excitation is caused by selective absorption, by the atoms or molecules to be analyzed, of electromagnetic radiation, this represents a *fluorescence* emission (or a *phosphorescence* emission, depending on the electron excitation state involved)

As with absorption, fluorescence may be applied, in the UV-visible radiation region, to molecules, or atoms. X-ray fluorescence spectrometry, on the other hand, refers to the X radiation emitted by atoms excited by absorption of X-radiation. Fluorescence techniques are more complex to implement than is the case for absorption techniques, since they entail that the particle subjected to analysis be selectively excited by a monochromatic radiation. On the other hand. since the radiation emitted is likewise specific to the particle, fluorescence spectrometry involves a double selectivity, resulting in very low background noise, thus making it peculiarly well suited for the measurement of very low concentrations.

Emission of radiation may also occur when atoms are thermally excited, in an environment brought to high temperatures. Emission spectroscopy is based on the fact that atoms, or molecules excited to high energy levels deexcite to lower levels, by emitting radiation (emission, or luminescence). This differs from fluorescence spectrometry in that excitation is not applied selectively, rather it involves indiscriminately all of the particles making up the medium. Emission lines thus correspond to radiation directly emitted by a body brought to a high temperature, and the emission spectrum allows the detection, and quantification, of all atoms present in the emission source.

Raman spectrometry

Interactions between matter and electromagnetic radiation also give rise to scattering processes, such as elastic scattering, and inelastic scattering. Scattering may occur when the interface between

two media is encountered, or as a medium is passed through. This process, in most cases, is an "elastic" one, in other words it takes place with no change in frequency for the radiation forming the beam involved. Elastic scattering of solar radiation by the atmosphere is, for instance, responsible for the blueness of the sky, observed when the eye is not directed towards the Sun (*Tyndall effect*). Indeed, scattered intensity is all the greater, the shorter the radiation wavelength, which, in the case of the solar spectrum, corresponds to the color blue.

As regards spectrometry, the main use of scattering concerns Raman spectrometry. This involves the inelastic scattering of incident radiation by the molecules making up the sample. The difference between scattered radiation frequency, and incident radiation frequency allows the identification of the chemical bonds involved. Raman spectrometry is a technique that is widely used for structural analysis, to complement infrared spectrometry, and mass spectrometry.

Nuclear magnetic resonance spectrometry

The principle of nuclear magnetic resonance (NMR) is based on the fact that an atom has a magnetic moment, just like a spinning charge acting as a tiny magnet, governed by quantum mechanics, aligning in a magnetic field as the needle of a compass in the Earth's magnetic field. The principle of NMR consists in inducing, and detecting, the transition, for the nuclear magnetic moment, from the lowest energy level to the highest energy level, through absorption of electromagnetic radiation of a wavelength lying in the radiofrequency region: when the energy of the photon precisely matches the energy difference between the two levels, absorption occurs. Nuclei having numbers of protons, and neutrons that are both even exhibit zero spin. Carbon 12 and oxygen 16 atoms, which are very widespread in nature, thus have zero spin. On the other hand, hydrogen only has one single proton, and its nuclear magnetic moment equals 1/2: it may thus take on two possible energy states, corresponding to the two orientation states of its spin, relative to the magnetic field. Measuring the resonance frequency in the electromagnetic field allowing transition from one of these energy states to the other enables the molecu-



Spectromètre de masse d'ions secondaires utilisé au CEA pour réaliser des mesures isotopiques rapides sur un échantillon par exemple prélevé sur une installation aux activités nucléaires suspectes.

les to be analyzed. This frequency is fixed, however the various nuclei in a molecule do not all resonate at the same frequency, since their magnetic environment is modified by their chemical (electronic) environment

Many NMR spectra exhibit more peaks than there are protons in the nucleus, owing to the interactions between protons and their neighbors. Two nuclei may interact within the molecule, though they are separated by several chemical bonds: this is known as interatomic coupling. This interaction endows the NMR spectrum with a fine structure.

Mass spectrometry

Mass spectrometry is a highly sensitive detection and identification technique, allowing determination of molecular structures, and thus of a sample's composition. This is not, strictly speaking, a form of spectrometry, since it is not concerned with discrete energy levels. What is its principle? A compound introduced into the device is vaporized, and subsequently ionized by an electron bombardment source (at 70 eV). The ion thus obtained, termed a molecular ion, allows the compound's molar mass to be determined. Breaking chemical bonds within the compound may yield characte-

ristic fragment ions. These are then sorted according to their mass/charge ratio in an analyzer, through application of a magnetic and/or electric field, then collected by a *detector*, which amplifies the signal associated to the ions, which arrive with varying delays. A data processing system converts the information from the detector into a mass spectrum, readout of which, by comparing it with reference spectra, allows the identity details of the molecule to be drawn up. Through use of a highresolution mass spectrometer, the exact mass of the compound may be determined, together with isotope percentages for each constituent atom.

Choice of ionization method is directly related to the nature of the sample, and the type of analysis. If mass spectrometry has gradually adapted to meet the growing demands from chemists, and biologists (separation of increasingly complex, highly polarized mixtures, determination of ever higher molecular masses on samples of ever more constricted sizes), this is essentially due to advances in ionization techniques, these including secondary ion mass spectrometry (SIMS), chemical ionization, thermospray ionization, and fast atom bombardment (FAB) sources, further comprising, from the 1980s, matrix-assisted laser desorption ionization (MALDI), and electrospray ionization (ESI), together with advances in detection techniques, from time-of-flight (TOF) measurement to "ion traps" (ITs), through quadrupoles (MS or Q).

In proteomics, for instance, only MALDI, ESI and SELDI (surface-enhanced laser desorption ionization) are employed.

Ion mobility spectrometry (IMS) is a chemical analysis technique in the gaseous phase, which consists in subjecting a gas to an electric field. Ionized molecules acquire a velocity that is characteristic for the ion, since this depends on mass, and charge. Arrival of the ions on one of the plates generating the field results in a current, which is recorded. The length of time after which a peak occurs can be related to the nature of the ion causing it.

Scientists often make use of a coupling of devices each belonging to one of the two main families of analytical techniques (see Box E, What is chromatography?), e.g. of a chromatograph with a mass spectrometer (or an electron-capture detector [ECD]), particularly for the investigation of trace complex mixtures.

Fundamental interactions and elementary particles

he standard model of particle physics is the reference theoretical framework describing all known elementary particles (see Table 1) and the fundamental interactions these particles are involved in (see Table 2). The basic constituents of matter, known as fermions, are partitioned into two main categories, as determined by their partcipation in the fundamental interactions, or forces (the gravitational, electromagnetic, weak, and strong forces), which are mediated by vector bosons, the fundamental particles which carry out the transmission of the forces of nature^[1] (see Table 2). Whether a particle belongs to the category of fermions, or to that of bosons depends on its spin (i.e. its intrinsic angular moment, or internal rotation moment), depending on whether it exhibits half-integer spin (fermions) or integer spin (bosons).

At the same time, to every constituent of matter is associated its **antiparticle**, a particle having the same *mass*, but the opposite *charge*. The **positron** is thus the positively charged antiparticle of the **electron**, which exhibits a negative charge.

Leptons and quarks

Fermions include, on the one hand, leptons, which may travel freely and do not participate in the *strong interaction*, which ensures the cohesion of atomic nuclei (it is consequently termed a *nuclear* interaction), and, on the other hand, quarks, which participate in all interactions but are not individually observed, enmeshed and confined as they are within hadrons, the particles susceptible to strong interaction, of which they are the constituents.^[2]

In the lepton category, charged leptons participate in the electromagnetic interaction (which ensures the cohesion of atoms and molecules, and in the weak interaction (which underlies decay processes, in particular β radioactivity). Neutral leptons, or neutrinos, for their part, participate in the weak interaction only. Exhibiting very low mass, there is one type of neutrino for each type of charged lepton.

Independently from their involvement in interactions, the basic constituents of matter are classified into three *gene*-

rations, or families, of particles. From one family to the next, quarks and leptons having the same charges only differ by their mass, each family being heavier than the preceding one.

The electron, up quark (symbolized *u*) and down quark (symbol *d*), which belong to the first generation, are the lightest massive particles, and are stable. These are the sole constituents of normal matter, so-called baryonic matter (a baryon is an assembly of quarks), which is made up of protons and neutrons, this however only accounting for 4% of the Universe's energy content! Particles in the other two families are heavier, and are unstable, except for neutrinos, which on the other hand exhibit non-zero mass, but are stable.

These latter particles may only be observed or detected in the final states resulting from collisions effected in accelerators, or in cosmic radiation, and rapidly decay into stable first-generation particles. This is why all the stable matter in the Universe is made up from constituents from the first family. According to quantum mechanics, for an interaction to take place between particles of normal matter, at least one elementary particle, a boson, must be emitted, absorbed, or exchanged. The photon is the intermediate (or vector) boson for the electromagnetic interaction, the W+, W- and Z are the intermediate bosons for the weak interaction, and gluons are those of the strong interaction, acting at quark level.

As to the **graviton**, the putative vector for the gravitational interaction, it has not so far been empirically discovered. The **gravitational force**, which acts on all fermions in proportion to their mass, is not included in the standard model, due in particular to the fact that quantum field theory, when applied to gravitation, does not yield a viable scheme, as it stands. While gravitational effects are negligible in particle physics measurements, they become predominant on astronomical scales.

Interaction ranges

Quarks and charged leptons exchange photons. The photon having no electric charge, these particles conserve their electric charge after the exchange. Since the photon's mass is zero, the electromagnetic interaction has an infinite range. Having no electric charge, neutrinos are the only elementary fermions that are not subject to electromagnetic interaction.

In the electroweak theory (a unification of the weak and electromagnetic interactions), the weak interaction has two aspects: charged-current weak interaction, for which the interaction vectors are the W⁺ and W⁻; and neutral-current weak interaction, for which the mediator is Z⁰. These two forms of weak interaction are active between all elementary fermions (quarks, charged leptons and neutrinos). The mass of these bosons being very large (about 80 GeV/c2 for W^{\pm} , 91 GeV/ c^2 for Z^0), the range of the weak interaction is tiny - of the order of 10-18 m. Since W[±] bosons have a nonzero electric charge, fermions exchanging such bosons undergo a change in electric charge, as of nature (flavor). Conversely, since the Z⁰ boson has no electric charge, fermions exchanging one undergo no change in nature. In effect, neutral-current weak interaction is somewhat akin to exchanging a photon. As a general rule, if two fermions are able to exchange a photon, they can also exchange a Z^0 . On the other hand, a neutrino has the ability to exchange a Z⁰ with another particle, though not a photon.

Only those quarks that have a color charge⁽¹⁾ exchange gluons, these in turn being bearers of a color charge. Thus,

- (1) The participation of basic constituents in fundamental interactions is governed by their interaction charges (electric charge, color charge), or "conserved quantum numbers." Color charge, a quantum number that determines participation in strong interactions, may take one of three values: "red," "green," or "blue" (these colors bearing no relation to visible colors). Every quark bears one of these color charges, every antiquark one of the three anticolor charges. Gluons are endowed with double color—anticolor charges (eight combinations being possible).
- (2) To take e.g. nucleons: the proton holds two up quarks and one down quark, the neutron two down quarks and one up quark. A meson is made up of just two quarks (one quark and one antiquark).

Gont'd)

when a gluon exchange takes place between quarks, the latter exchange their respective colors. Gluons have zero mass, however, since they do bear a color charge, they are able to interact together, which greatly complicates theoretical treatment of this interaction. The range of the strong interaction is consequently very restricted - of the order of 10^{-15} m.

The quest for unification

The theoretical framework for the standard model is quantum field theory, which allows a quantitative description to be made of the fundamental interac-

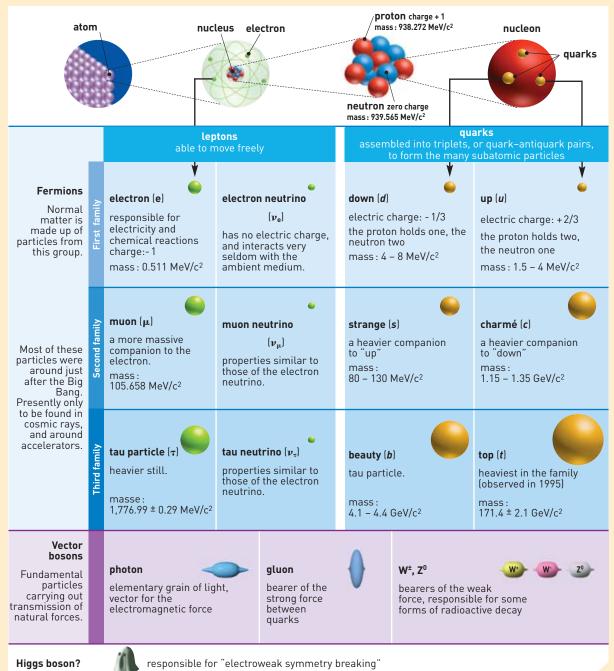




Table showing the twelve elementary constituents for which the standard model describes the interactions involved. The three charged leptons [electron e-, muon, μ -, tau particle τ -] are subject to electromagnetic and weak interactions, neutrinos $\{\nu_e, \nu_\mu, \nu_\tau\}$ are only affected by weak interaction, and the six quarks (up, charm, top – or u, c, t – bearing a charge of 2/3; and down, strange, bottom – d, s, b – bearing a charge of - 1/3) are subject to all three interactions. Every elementary constituent has its antiparticle, having the same mass, and algebraic quantum numbers (such as electric charge) of the opposite sign.

tions between elementary particles, while respecting the principles of special relativity, as those of quantum mechanics. According to the latter theory, if one seeks to observe a microscopic structure at high temporal and spatial resolution, this entails transferring to it an amount of energy-momentum, the greater, the higher the resolution being sought. However, according to the theory of relativity, such an energy-momentum transfer is liable to undergo transformation, yielding particles not present in the initial state: fermions may be generated, or annihilated, in particle-antiparticle pairs, while bosons may be so in any arbitrary number.

All processes involving one and the same fundamental interaction are interrelated. The quantum field theory approach, in which properties of symmetry play a fundamental part, seeks to describe all of the processes relating to each fundamental interaction, within overarching theoretical constructions.

The strong and electromagnetic interactions are formalized, respectively, in the theories of quantum chromodynamics, and quantum electrodynamics. The weak interaction, for its part, is not subject to a separate description, being described jointly with the electromagnetic interaction, in the unified formalism of electroweak theory. Theories of the grand unification of all fundamental interactions do exist, however they remain as yet lacking any experimental validation.

All the predictions of the standard model have been corroborated by experiment, except for just one, to wit, the existence of the Higgs boson(s), which particle (particles?), it is hoped, will be discovered with LHC. The Higgs mechanism is thought to be responsible for the mass exhibited by elementary particles, the eponymous boson making it possible for zero-mass fermions interacting with it to be endowed with mass. This would allow the unification, at high energies, of the weak and electromagnetic interactions within the electroweak theory, while effectively accounting for the breaking of this electroweak symmetry at low energies, taking the form of two interactions, which may be seen as distinct at that energy level (see *The electroweak*

interaction from one accelerator to the next: the LHC roadmap and the yardstick of LEP measurements, p. 23).

Going beyond, or completing the standard model?

The standard model features a set of parameters (such as the masses of elementary particles, or the intensities of fundamental forces) which are "anchored" in experimental findings. It is, in any event, a theory that is liable to be improved, or further elaborated, or even surpassed and left behind. It does not account in any way for the classification of the constituents of matter into three generations of particles, whereas it is precisely the existence of these three generations which makes it possible to account for CP (charge-parity) invariance violation (meaning that a physical process involving the weak interaction is not equivalent to its own mirror image), a violation that is in all likelihood the source of the matter-antimatter imbalance, running in favor of the former, in the primordial Universe. The model neither allows quantum treatment of gravitation, nor does it fully account for the fundamental property of confinement, which prevents quarks from propagating freely outside hadrons.

To go beyond, or to complete the standard model, research workers are mainly exploring two avenues:

- supersymmetry (widely known as

SUSY) would associate, to every particle (whether a boson or a fermion) in the standard model, a partner from the other series, respectively a fermion or a boson. Supersymmetric partners would, at first blush, be highly massive, the lightest of them being a particle interacting very weakly only. This would be an ideal candidate to account for the hidden matter (or dark matter) in the Universe, accounting as it does for some 21% of the Universe's energy content, the remainder (close to 75%) consisting in a dark energy, the nature of which likewise remains to be determined. These WIMPs (acronym for "weakly interacting massive particles") are actively being sought (see EDELWEISS II, the guest for dark matter particles);

- the **substructure** path assumes there could be a new level of elementarity, underlying the particles in the standard model (or some of them). This would lead to a veritable blossoming of new, composite particles, analogous to hadrons, but exhibiting masses two to three thousand times heavier.

It should be noted that, whereas supersymmetry theories yield predictions that agree with the precision measurements carried out at LEP, the theories propounding substructures (or their simpler variants, at any rate) fail to do so. As for the more complex variants, these are encountering difficulties at the theoretical level.

fundamental interaction	associated particles (messengers)	actions
gravitation	graviton?	having an infinite range responsible for the mutual attraction of any two masses and for the law of falling bodies
electromagnetic interaction	photon	having an infinite range responsible for the attraction between electrons and atomic nuclei, hence for the cohesion of atoms and molecules
weak interaction	W+, W-, Z ⁰	responsible for β ⁻ and β ⁺ radioactivity, reactions involving particles as neutrinos
strong interaction	gluons (there are 8 gluons)	ensures the cohesion of the atomic nucleus

Tableau 2.
Fundamental interactions, their vectors, and effects.