Liquid and solid scintillation: principles and applications

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Abstract. Scintillation counting is one of the most important developments in the application of radioisotopes to procedures needed by scientists, physicians, engineers, and technicians from many diverse discipline for the detection and quantitative measurement of radioactivity. In fact, Scintillation is the most sensitive and versatile technique for the detection and quantification of radioactivity. Particularly, Solid and Liquid scintillation measurement are, nowadays, standard laboratory methods in the life-sciences for measuring radiation from gamma- and beta-emitting nuclides, respectively.   This methodology is used routinely in the vast majority of diagnostic and/or research laboratories from those of biochemistry and biology to clinical departments.

1. Introduction

Radioactive isotopes of common elements are extremely useful in life science disciplines, among others, because radioactive atoms can be substituted for their non radioactive counterparts in chemical formulations. The resulting radioactive compound is easily detectable but still chemically identical to the original material.

Two different systems of detection and counting of radiolabeled compounds based on the scintillation technique have been developed: Solid Scintillation Counting (SSC) and Liquid Scintillation Counting (LSC) depending on the scintillator material used.

The most common scintillator materials are normally classified according to their nature. First, in SSC, the most used scintillator are crystals of inorganic material such as alkali halide crystals, mainly sodium iodide. The high atomic number and density of certain inorganic crystals make them suitable for gamma spectrometry with high-detection efficiencies. On the other hand, scintillators used in LSC tend to be either a liquid (organic solvents) or in solid form (plastics) and they are preferred for the detection of beta particles and neutrons.

In fact, the wide popularity of LSC is a consequence of numerous advantages, which are high efficiencies of detection, improvements in sample preparation techniques, automation including computer data processing, and the spectrometer capability of scintillation analyzers permitting the simultaneous assay of different nuclides.

Herein we summarize the basic principles of scintillation counting with examples of applications in biomedical and environmental sciences developed at the University of Barcelona with the support of Scientific and Technological Centers (CCiTUB) of this university.

2. Methodology

2.1. Physical principles of scintillation technique

Radioactive decay occurs with the emission of particles or electromagnetic radiation from an atom due to a change within its nucleus. Forms of radioactive emission include alpha particles (α), beta particles (β) and gamma rays (γ). Alpha & beta particles directly ionize the atoms with which they interact, adding or removing electrons. Gamma-rays cause secondary electron emissions, which then ionize other atoms. However, some irradiated atoms are not fully ionized by collision with emitted particles, but instead have electrons promoted to an excited state. Excited atoms can return to their ground state by releasing energy, in some cases as a photon of light. Such scintillation phenomena form the basis of a set of very sensitive radiation detection systems. To a first approximation this is a linear conversion of energy into photons and, therefore, the intensity of light in the scintillation is proportional to the initial energy deposited in the scintillator by ionizing radiation. This light emitted is taken as a measure of the amount of radioactivy in the sample [1].

2.2. Instrumentation. Scintillation counter apparatus

A scintillation counter measures ionizing radiation. A scintillation counter apparatus consists of a scintillator, a photo-multiplier tube (PMT), an amplifier, and a multichannel analyzer (Fig. 1).

A solid scintillation counter is aradiation detector which includes a scintillation crystal to detect radiation and produces light pulses while the liquid scintillation counter detect the scintillation produced in the scintillation cocktail by radiation.

The PMT is an electron tube that detects the blue light flashes from the scintillation and converts them into a flow of electrons and subsequently measured as an electric pulse. This consists of a photocathode (photoelectric emitter) that is connected to the negative terminal of a high tension battery. A number of electrodes called dynodes are arranged in the tube at increasing positive potential. When a scintillation photon strikes the photocathode of the PMT is released a photoelectron. Using a voltage potential, the electrons are attracted and strike the nearest dynode with enough energy to release additional electrons. The second-generation electrons are attracted and strike a second dynode, releasing more electrons. This amplification continues through 10 to 12 stages. More electrons are emitted and the chain continues, multiplying the effect of the first

charged particle. By the time the electrons reach the last dynode, enough have been released to send a voltage pulse across the external resistors. The magnitude of the resulting pulse height produced by the PMT is proportional to the photon intensity emitted by the scintillator (crystal NaI(Tl) in SSC or "cocktail scintillator" in LSC). This voltage pulse is amplified and recorded by a multichannel that classifies each voltage pulse. Pulses are collated into channels, and the counts per minute (CPM) in each channel is recorded. Each channel corresponds to a specific range of energies (channels are also known as counting windows), and counts with energies above or below set limits are excluded from a particular channel. When the counts have all been collated, the researcher knows the intensity of radiation, expressed as CPM, and its energy distribution, or spectrum. CPM is proportional to the amount of isotope in the sample, and the spectrum indicates the identity of the isotope.
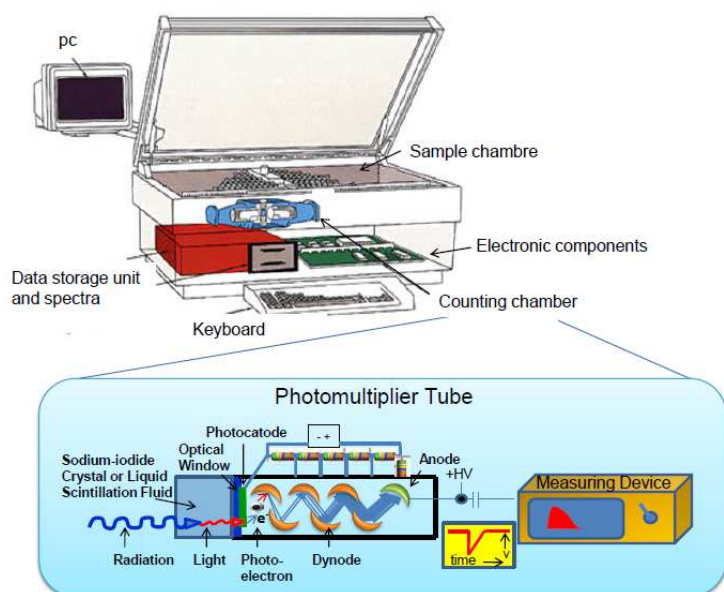
Figure 1. Illustration of a scintillation counter.

2.3. Mechanism of Solid and Liquid Scintillation Counting

In SSC, the transparent inorganic crystal, called scintillator, fluoresces when is irradiated by the sample (Figure 2 right). The most commonly used is Thallium-doped sodium iodide (NaI(Tl)). This detector is made of various sizes for different types of equipment. With this method, involves placing the sample containing the radioactivity into a glass or plastic container, called a scintillation vial that is deposited directly onto a solid scintillating material, dried, and counted in a scintillation counter also called Gamma counter [2].

Solid scintillation is excellent for γ radiation which is highly penetrating and interact with the NaI(Tl) detector by photoelectric, Compton and pair production mechanisms result in light or scintillations throughout a large crystal. An advantage of these techniques is that the same crystal is used for each sample, which enhances reproducibility. NaI(Tl) can be produced in large crystals, yielding good efficiency and producing intense bursts of light compared to other spectroscopic scintillators. Thus, NaI(Tl) is also suitable for use, making it popular for field applications such as the identification of unknown materials. However, because of the poor resolution of NaI-based detectors, they are not suitable for the identification of complicated mixtures of gamma ray-producing materials.

For α or β radiation counting, however, solid scintillation has severe limitations. The crystal must be protected from contamination by the sample, which means that the α and β particles must traverse a barrier prior to reaching the scintillator. In particular, α-radiation is severely attenuated by even 0.05 mm of aluminium or copper, and so cannot be expected to reach a scintillator crystal through even the thinnest shielding [1].

Crystal of zinc sulphide activated with silver, ZnS (Ag) is used in form of microcrystals pressed for the detection of heavy-charged particles (alpha, protons, fission products, etc.). In the case of alpha particles, the crystal mass thickness is about 5mg/cm^2, roughly equivalent to the scope of the alpha particles emitted by natural radionuclides. Because of its small thickness, this detector is very insensitive to beta particles or gamma radiation, property that is very useful when counting alpha particles in an intense background of beta particles or gamma photons.

In LSC, the samples are dissolved or suspended in a solution of organic liquid or cocktail scintillator (which is the sensor of the system) and the scintillation process takes place in a solution of scintillator rather than in a solid crystal. This allows close contact between the radioisotope atoms and the scintillator to assure efficient transfer of energy between the radioactive particles and the solution. Particularly, LSC is a standard laboratory method in the life-sciences for measuring radiation from beta-emitting nuclides [3].

Liquid scintillation cocktails absorb the energy emitted by beta particle and re-emit it as flashes of light (Figure 2 left) through two basic components, the aromatic solvent and small amounts of other additives known as fluors, i.e., scintillants or scintillators. In the first step, beta particles emitted from the sample transfer energy to the solvent molecules, which in turn transfer their energy to the fluors; the excited fluor molecules dissipate the energy by emitting photons of visible light (fluorescence). In this way, each beta emission (ideally) results in a pulse of light. The total number of photons from the excited fluor molecules constitutes the scintillation.

Some beta emitting isotopes (e.g. ^{32}P) can be analyzed on an LSC without using any cocktail, using a technique called Cherenkov counting. Cherenkov is based on charged particles passing through a transparent medium faster than the speed of light relative to the medium they are traversing (e.g., water, etc.). Cherenkov radiation (i.e., light), is produced being detected directly by the photomultiplier tubes.

In LSC, when a charged particle strikes the scintillator, a flash of light is produced, which may or may not be in the visible region of the spectrum, typically with low intensity in the ultraviolet region. Scintillation cocktails often contain additives that shift the wavelength of the emitted light to make it more easily detected. Many cocktails contain additional materials to extend their range of use to different sample compositions, but the solvent and the phosphor provide the scintillation of the mixture.
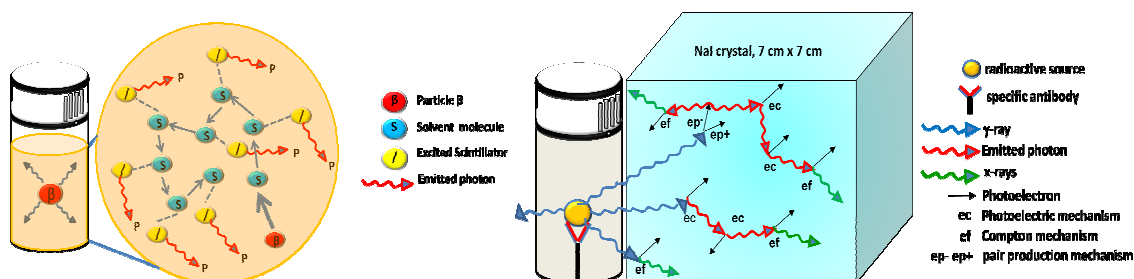
Figure 2. Left: Liquid Scintillation mechanism. Right: Solid Scintillation mechanism

2.4. Detection of interferences in solid and liquid scintillation counting

Solid scintillation gamma counting does not have the disadvantages of quenching normally associated with the liquid scintillation counting technique. This is because the sample is kept physically separated from the scintillator, which prevents any possible physical or chemical interference with the γ-energy transfer and scintillation processes.

In LSC there is a release of energy from the sample as photons, which is not due to the phenomenon of scintillation. This energy unduly increases the count or gives light pulses even in the absence of the radioactive sample producing interferences in the detection process. These must be eliminated from the sample or discriminated by the detection system. These interferences can be distinguished according to their origin:

- Chemiluminescence. It is the production of light as a result of a chemical reaction between components of the scintillation sample in the absence of radioactive material. This most typically occurs when samples of alkaline pH and/or samples containing peroxides are mixed with emulsifier-type scintillation cocktails, when alkaline tissue solubilizers are added to emulsifier type scintillation cocktails, or when oxidizing agents are present in the sample. Reactions are usually exothermic and result in the production of a large number of single photons. It has a slow decay time (from 0.5 hr to > 1 day, depending on the temperature) [4].

- Photoluminescence. Results in the excitation of the cocktail and/or vial by UV light (e.g., exposure to sunlight or UV lights laboratory). It decays more rapidly (usually < 0.1 hr).

- Quench. It is a reduction in system efficiency as a result of energy loss in the liquid scintillation solution. Because of quench, the energy spectrum detected from the radionuclide appears to shift toward a lower energy. The three major types of quench encountered are photon, chemical, and optical quench. Photon quenching occurs with the incomplete transfer of beta particle energy to solvent molecules. Chemical, sometimes called impurity, quenching causes energy losses in the transfer from solvent to solute. Optical or colour quenching causes the attenuation of photons produced in solute. This interference can be overcome through data correction or through careful sample preparation [4].

2.5. Quantitative analysis

In LSC, common nomenclature expresses the intensity of radiation emitted as disintegrations per minute (DPM, activity sample). The sample count rate depends on how efficiently nuclear decay events are converted to light flashes that are detected and quantified by the LSC [4].

Because the sample solution is always present, it can absorb nuclear decay energy thereby preventing this energy from being absorbed by the chemical fluorine molecules, or the solution can absorb photons of light that are emitted by the scintillation cocktail. This causes the phenomenon called quench defined as interference with the conversion of decay energy to photons emitted from the sample vial. In order to compensate for quench and determine the DPM (actual activity sample) from CPM (counts per minute measured), it is necessary to know the counting efficiency: % Eff = (cpm/dpm) x100

The lower the radiation energy of the radionuclide, the greater is the effect of quench on the counting efficiency of the sample. Techniques have been developed for applying these corrections, and a great deal of research has been carried out to improve the efficiency of counting, using various detection systems: internal standardization, sample spectrum quench correction, external standard quench correction, Direct DPM methods [5].

3. Applications

Solid and liquid scintillation techniques are used for the detection of radio labeled isotopes in areas as diverse as biomedicine, ecology and industry.

Scintillation counting capabilities include detection of alpha, beta and gamma emitters, in single, double and triple labelling, and also include the detection of these transmitters by counting in continuous flow (HPLC) and finally the scintillation proximity Assays (SPA)

Detecting and counting alpha emitting radionuclides are routine tasks in nuclear energy and environmental monitoring. Liquid scintillation counting of alpha particles provides high counting efficiency (near 100%). The accurate and sensitive measurement of alpha-emitting nuclides is essential in the nuclear fuel cycle, process control, radioactive waste management and environmental protection. However the energy resolution is quite poor, so they are not very useful for the identification of these radionuclides.

In detection of beta emitters, LSC is the most important application of scintillation counting, with the property that for high-energy beta emitters (^{89}Sr, ^{90}Y, ^{32}P) performance may be close to 100%, while for low energy is lower: 50-60% for ^{3}H, 95% for [14]C. However, it is often the

technique of choice for these weak beta emitters. LSC is extensively used, for in vivo and in vitro biomedicine research. The usefulness of radioisotopes in this research stems from their chemical identity with their non-radioactive counterparts. This allows their incorporation into "tracers", radiolabeled components which can be followed and detected through a series of reaction steps. Some of these studies include from carbohydrate [6, 7] and lipid [8, 9] metabolism assays, enzyme activity determination [10, 11], hormone studies [12, 13] to the amino acid [14] and nucleoside transport studies [15, 16, 17]. On the other hand, experimental procedures seek screening of peptide library with Radioligand binding assays [18]. Certain genetic and biochemical studies use high-energy beta emitters as in the case of ^{45}Ca, ^{32}P and ^{22}Na, which are used to quantify the LCS [19, 20, 21]. Other applications are radioactivity contaminant detections for environmental monitoring and ecology studies such as bacterial or microbial activity [22, 23].

Moreover, some beta-emitting isotopes can be analyzed on an LSC without using any cocktail or with only a little water, using a technique called Cherenkov counting [24].

Detection of gamma particles is of special interest in clinical and basic biomedical research. We noted several studies: Hormone determination in blood tests [12], analytical practice for hormone levels determination by radioimmunoanalisis techniques [25] and identification of different radionuclide contaminants in soil samples [26, 27, 28].

In this section we show some examples of applications of liquid and solid scintillations which briefly illustrate the capabilities of these techniques:

3.1. Cell Proliferation assays

Cell proliferation assays measure the incorporation of a radiolabeled DNA precursor, ^{3}H- or ^{14}C-Thymidine, into the replication strands of DNA produced during cell division. Cell proliferation studies based on the thymidine incorporation assay are employed frequently in immunological, cancer, stem cells, and pharmaceutical research to assess the ability of both, natural and synthetic compounds, to stimulate or inhibit cell proliferation.

Before a cell divides, its DNA is replicated and precursors are incorporated, thus if the cells are proliferating and [^{3}H]-Thymidine is added to the culture, it will be incorporated into the cells' DNA. The amount of [^{3}H]-Thymidine incorporated into the DNA is measured with a scintillation counter. The level of the radioactive signal depends on the proliferation rate.

Usually, an inhibitor of cell proliferation is added to the culture, in its presence the proliferation inhibition is calculated from the following expression: [%] of proliferation inhibition = [cpm (untreated) - cpm (treated)] / cpm (untreated)

The ^{3}H-Thymidine incorporation assay is commonly used to measure the effect of a pharmacological or drug treatment on proliferation of cultured cells [29], also to check an effect of cytotoxic drugs on tumor cells or carcinogenetic processes [30], to determine the cell cycle and its phases, to measure the rate of DNA synthesis or to detect antigen-specific T-cell proliferation in culture, among others.

3.2. Metabolites transport determination

The plasma membrane is a selectively permeable barrier between the cell and the extracellular environment. This permeability ensures that essential molecules such as glucose, amino acids, and lipids readily enter the cell, metabolic intermediates remain in the cell, and waste compounds leave the cell. In short, the selective permeability of the plasma membrane allows the cell to maintain a constant internal environment [31].

Transporters through conformational changes expose binding sites to their specific ligands that can join and be transported across the lipid bilayer of the cell membrane [32].

Different radiolabeled molecules are used in a wide range of metabolite transport applications from a simple study of its transport into the cell to more complete studies such as analysis of their metabolic pathways. Transport experiments can be carried out both in vivo(e.g. pumping substances through cell membranes, analyzing glycolysis pathways with ^{14}C-glucose) and in vitro(e.g. analysis, identification and / or quantification of enzyme activity).

Metabolite transport methodology in vitro is based on the incubation of cultured cells with a medium containing a radioactive substrate which will be incorporated to the cell. Briefly, after incubation cells are washed to eliminate the radioactive compound still present in the culture medium and then a cell lysate is obtained for each sample. Samples are mixed with the scintillation cocktail and counted in a LSC.

Herein we describe in detail three kinds of metabolite transport studies commonly assayed in our radioactive installation laboratories. These are: nucleoside transport, glucose transport and amino acid transport.

3.2.1. Nucleoside transport

The nucleotides are of great importance in cell physiology, since they constitute the structural elements of nucleic acids and are therefore essential for cell viability. Also, they intervene in energy homeostasis of the cell due to their involvement in many biochemical processes such as energy metabolism. The nucleosides can act as facilitators of a wide variety of specialized functions and play an important role in human physiology.

Different radiolabeled substrates can be used in nucleoside transport analysis such as the nucleosides: [3H] Uridine, [3H] Guanosine, [3H] Cytidine and [3H] Adenosine and nucleosides analogues: [3H] Gemcitabine, and [3H] Fludarabine [15, 33, 34, 35]. These are used in studies of drug resistance, cancer therapy and chemotherapy. Also, nucleoside reverse transcriptase inhibitors (eg. [3H] MPP+(metformin), [3H]3TC(lamivudine), [3H]ABC(abacavir) and [3H]AZT (azidothymidine)) can be used as radiolabeled substrates in "Short-Time" Uptake measurements, to determine their IC_{50} values [36].

Nucleoside transport methodology in monolayer cultured cells involves incubation of cells with a known concentration of cold substrate to study, to which an appropriate proportion of the radiolabeled substrate is added [37]. Once the cells have been incubated for the time required for testing, the uptake is stopped by washing and a cell lysate is obtained for each condition. Each sample is measured in a LSC in order to quantify the radioactivity retained which corresponds to the amount of substrate incorporated into the cell.

The nucleoside adenosine is among the most studied. Its interaction with membrane receptors maybe involved in the regulation of a variety of physiological processes among them, neurotransmission, cardiovascular activity, lipolysis or platelet aggregation (Figure 3A). In these processes, adenosine plays a role in cell protection (Figure 3B) [38].
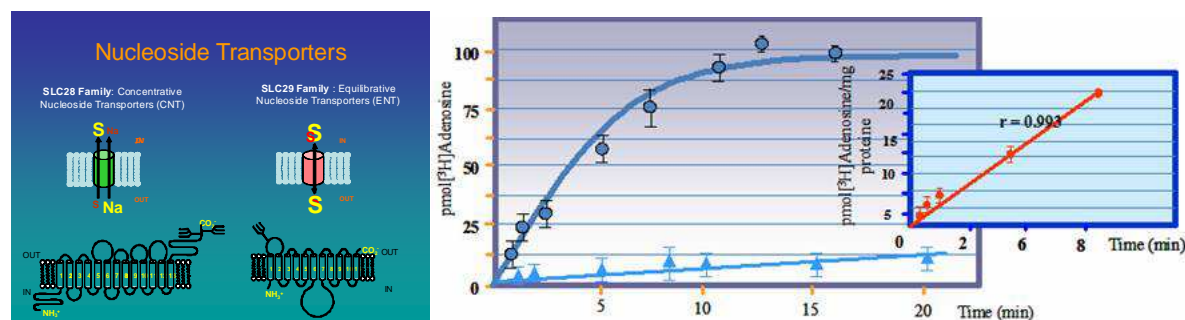

Figure 3: A) Image showing the two nucleoside transporter families. The nucleosides are relatively hydrophilic molecules, thus its internalization into cells is dependent on specific membrane transporters (Image courtesy of Dra I. Huber). B) [3H] Adenosine accumulation by rat testis mitochondria as a function of incubation time.

3.2.2. Glucose transport

Glucose uptake experiments are commonly used to measure cellular metabolic activity and glucose transport. Glucose uptake can be studied using radiolabeled glucose itself or radiolabeled glucose analogous such as 2-deoxy-D-glucose (2DG) or 3-O-methyl-D-glucose (OMG). The most common technique is the use of radiolabeled 2-deoxy-D-glucose, a glucose analog. Once 2-deoxy-D-glucose has been taken up by cells, it is phosphorylated and cannot be metabolized further. Labelled 2-

deoxy-D-glucose phosphate is trapped in the cell (unidirectional transport). By contrast, 3-O-methyl-D-glucose is not phosphorylated, and equilibrates across the cell membrane. Because equilibrium is usually reached rapidly, uptake is typically linear for only a short period of time. D-glucose itself (as opposed to glucose analogous such as 2-deoxy-D-glucose or 3-O-methyl-D-glucose) can be incorporated into lipids, providing a measurement of glucose transport.

As an example, we highlight a recent study carried out in a stable 3T3L1 cell line with a reduced expression of Caveolin-1 where the impact of this reduced expression on insulin action in adipose tissue is evaluated. In this study, the radiolabeled products used are the non-metabolizable glucose analogue 2-deoxy-^{3}H-glucose (to measure glucose uptake) and ^{14}C-Glucose (Glycolysis) [7].

Another interesting example is the comparison of glucose oxidation rates (realized using [U-^{14}C]-glucose) in cardiac cells that over express PGC-1α or silence it after being treated with TNF-α. The purpose of this study is to elucidate the specific mechanisms by which exposure to tumour necrosis factor-a (TNF-α) results in PGC-1α down-regulation in cardiac cells and, as a consequence, in metabolic dysregulation that underlies heart dysfunction and failure (Figure 4) [39]
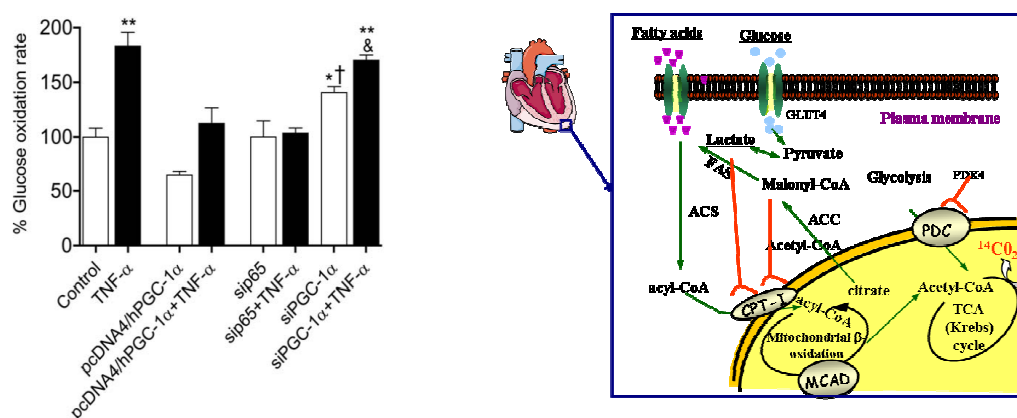
Figure 4 . The modulation of PGC-1α and p65 levels has a direct effect on glucose oxidation. The graph represents the [U-^{14}C]-glucose oxidation rates in control AC16 cells, and AC16 cells over expressing the human PGC-1α gene or with siRNA knock down of p65 or PGC-1α genes, and incubated with TNF-α. Thus, treatment with TNF-α induced the glucose oxidation rate up to 80% with regard to control cells and, as expected, down-regulation of PGC-1α expression with siPGC-1α increased the glucose oxidation rate up to 40%. The glucose oxidation rate was further increased when TNF-α was added to siPGC-1α compared with siPGC-1α alone (Adapted from [39]).

3.2.3. Amino acid transport

Amino acid transport across the plasma membrane mediates and regulates the flow of these ionic nutrients into cells and, therefore, participates in interorgan amino acid nutrition. In addition, for specific amino acids that act as neurotransmitters, synaptic modulators, or neurotransmitters precursors, transport across the plasma membrane ensures reuptake from the synaptic cleft, maintenance of a tonic level of their extracellular concentration, and supply of precursors in the central nervous system. Transfer of amino acids across the hydrophobic domain of the plasma membrane is mediated by proteins that recognize, bind, and transport these amino acids from the extracellular medium into the cell, or vice versa [40].

Functional studies based on saturability of transport, substrate specificy, kinetic behaviour, mode of energization, and mechanisms of regulation performed in perfused organs, isolated cells, and purified plasma membranes led to the identification of multiplicity agencies in the plasma membrane mammalian cells. In many of these studies, radiolabeled amino acids are used, as is the case of [^{3}H] Arginine [14, 41].

3.3. Radioimmunoassay

Radioimmunoassay (RIA) is a technique that allows the detection of very small quantities, of the order of nanograms, of biological or pharmacological substances, in blood or other fluid samples using antigen/antibody reactions. Among the most recent examples of RIA published by our researchers we emphasize those using ^{125}I-EGF [42], FGF21 [43] and 11-ketotestosterone and testosterone [13].

RIA is based on measuring the amount of radioactively-labelled antigen that is displaced from the specific antibody binding sites due to the arrival and subsequent unlabeled antigen competition (which is the unknown), knowing the amount of both, radioactively labelled antigen as unlabeled antigen in our sample. The measurement is made of the remaining free fraction before and after the addition of unlabeled antigen (Fig. 5).

The development of this technique offers several advantages over other detection methods. Sensitivity is the most important advantage as it is able to detect small amounts of substances as a result of the ability to measure very small amounts of radioactive tracers. A second advantage is the specificity, the ability of the technique to measure only the substance of interest in a sample that has a complex composition. The accuracy and reproducibility are others advantages since this technique allows to determine the effective amount of a substance and give highly reproducible measurements of concentrations in duplicate samples in a simple analysis.

The radioactivity of bound (cpm Bound) and unbound (cpm Free) components can be measured by standard techniques. The gamma counter is used in the case of those tracers that emit gamma rays, such as ^{125}I and [131]I. Due to the high specific activity of radiolabeled antigen or ligand, it is often easy to obtain high count values, allowing even greater statistical accuracy.
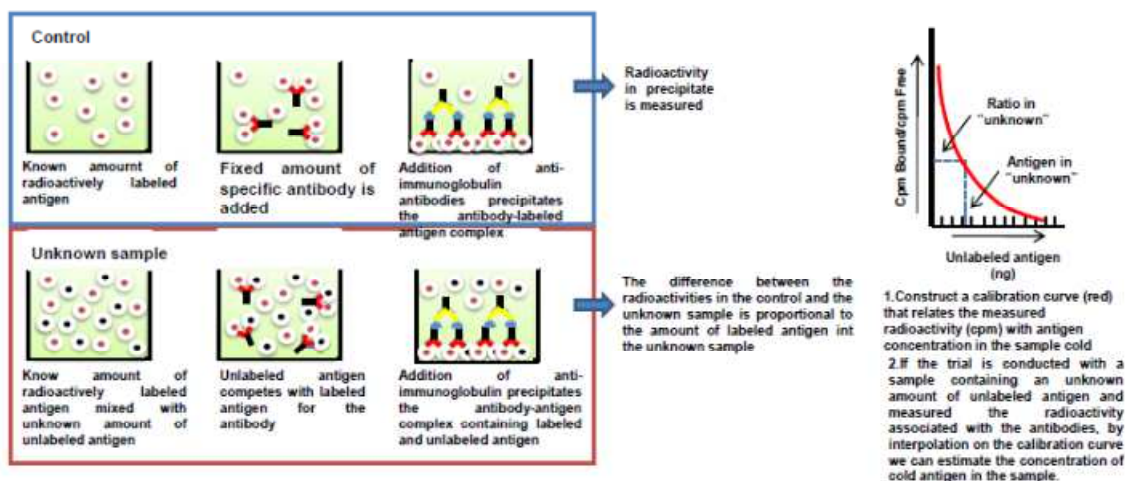
BT.12

Figure 5. Experimental procedure of a Radioimmunoassay

3.4. Environmental liquid scintillation counting

Environmental liquid scintillation counting (LSC) is the measurement of both natural and anthropogenic radionuclides in the natural environment, but often radionuclide concentrations are low. Many radionuclides are routinely measured at natural environmental levels in a range of sample matrices including waters [44], sediments, soils, air, etc. These include isotopes of radium (Ra), uranium (U), thorium (Th), ^{210}Pb, [222]Rn, and [231]Pa. Monitoring the environment for radionuclide releases is associated with nuclear fuel cycle activities (fuel enrichment, fuel fabrication, power generation, and fuel reprocessing facilities). This would principally be the analysis of beta-emitting radionuclides without significant gamma emissions including ^{3}H, ^{14}C, [36]Cl, [89,90]Sr, ^{90}Y, ^{99}Tc, and [241]Pu but could also include analysis of alpha emitting radionuclides. Studying the rates of processes in the environment would mainly be carried out using radionuclides of natural origins and would include ^{14}C dating, ground water movement and dating using ^{3}H, marine sediment mixing, productivity, and particle flux studies using [234]Th and ^{210}Pb/[210]Bi/[210]Po.

The photosynthetic activity of microorganisms in environmental samples can be determined by incubation with $NaH^{14}CO_3$ [22, 23].

3.4.1. Radiocarbon dating
The basis of radiocarbon dating technique is the relatively constant natural production rate of ^{14}C in the upper atmosphere, its uniform uptake as $^{14}CO_2$ into living plant material, conversion to plant carbohydrates, and subsequent transfer through the food chain. The end result of the food chain transfer is almost uniform labelling of all living organisms. ^{14}C dating is used in a wide range of scientific disciplines including geology, archaeological [45], mineral science, soil science, oceanography, climate reconstruction, etc. and LSC is commonly used for this measurements.

3.4.2. Food and additives studies
Petroleum derivatives are occasionally used to adulterate natural food and drink products without the buyer's knowledge. Because petroleum-based products are sufficiently old that they contain no ^{14}C, depletions in the natural ^{14}C content are normally indicative of adulteration.
Determination of $^{14}CO_2$ in biological samples, with the aim of quantifying total oxidation of oleic acid and glucose $^{14}CO_2$ is measured in different studies [8, 46].

Acknowledgements

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