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BIOMEDICAL ENGINEERING MASTER THESIS

PARTICLE-BASED (MICRO/NANO) BIOMATERIAL SYSTEMS FOR ION RELEASE

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ABSTRACT

In this work, we have compiled the information about particle-based (micro/nano) biomaterial systems for ion-release and their possible applications in the bio-sanitary field. In the last decades, nanoparticles and microparticles have been used as a vehicle for drug-release, however, scarce information describe particle-based material systems for ion-release as a therapeutic approach. This work will collect the present information concerning the applications of particle-based material systems for ion-delivery in general and in particular we will focus on boron ion. First, the work gathers a brief study about the particle-based methods for ion-release such as gold or silver ions. After that, a general description of boron and its uses in different industries is made, as well as its effect in different compounds and its participation in the plant and animal metabolism. Especial emphasis has been made in the applications for boron and its derivatives in medicinal chemistry. A general description of the different particle-based encapsulation systems that can be adjusted for boron-release is also described. Further, we introduce, the different methods used for detection of the released ions and their determination once introduced in organisms and cellular systems and analysed their traceability. Finally, we describe, the experimental approaches and applications performed for particle boron delivery, as well as recent studies describing boron homeostasis and putative mechanism of action in mammals, unknown to date. The purpose of this work is to collect the existent information about the application of particle-based systems for ion-release (especially boron), emphasising the role and the importance of these material systems will have in the next years for biomedical applications.

Keywords: boron, nanoparticles, ion-release, particles, encapsulation.

RESUMEN

En este trabajo se recogen la información sobre sistemas de biomateriales basados en partículas (micro/nano) para la liberación de iones y sus posibles aplicaciones en el ámbito biosanitario. Las nanopartículas y micropartículas como métodos de transporte y liberación de fármacos llevan utilizándose en las últimas décadas, sin embargo, la liberación de iones desde estos sistemas materiales con fines terapéuticos no ha sido tan estudiada y este trabajo recopilara información sobre el uso de estos, centrándose en el caso del boro. Primero, el proyecto recoge un breve estudio sobre el uso de métodos basados en partículas para la liberación de iones como los iones de oro o de plata. Después, se hará una descripción general del boro y el uso que se la da en las diferentes industrias, así como sus efectos en diferentes compuestos y seres vivos. Se hará un especial hincapié en las aplicaciones que el boro y sus derivados puedan tener en la química medicinal. Una recopilación general de los diferentes sistemas de encapsulación existentes (basados en partículas) que sean adaptables para el ion de boro también está presente en el trabajo. Del mismo modo, se analizan los diferentes métodos que se utilizan para la detección de iones una vez introducidos en los sistemas celulares y su trazabilidad. Por último, se analizan las aplicaciones y experimentos llevados a cabo en lo relacionado con la liberación de iones de boro mediada por sistemas materiales basados en partículas, tanto en casos ya demostrados hace tiempo como en los últimos estudios al respecto. La finalidad de este trabajo es recopilar la información existente sobre las aplicaciones de sistemas basados en partículas para la liberación de iones (especialmente del boro) y presentarla de una manera que facilite la comprensión de la importancia e impacto que estos sistemas tendrán en un futuro próximo para aplicaciones biomédicas.

Palabras Clave: boro, nanopartículas, liberación de iones, partículas, encapsulación.

RESUM

En aquest treball s'arreplega l'informació sobre sistemes de biomaterials basats en partícules (micro/nano) per a l'alliberament d'ions i les seues possibles aplicacions en l'àmbit biosanitari. Les nanopartícules i micropartícules com a mètodes de transport i alliberament de fàrmacs porten utilitzant-se en les últimes dècades, no obstant això, la càrrega d'ions amb finalitats terapèutiques no ha sigut tant estudiada i aquest treball recopila informació sobre l'ús d'aquestes, centrant-se en el cas del bor. Primer, el projecte arreplega un breu estudi sobre l'ús de mètodes basats en partícules per a l'alliberament d'ions com els ions d'or o de plata. Després, es farà una descripció general del bor i l'ús que se li dona en les diferents indústries, així com els seus efectes en diferents compostos i éssers vius. Es farà un especial recalcament en les aplicacions que el bor i els seus derivats puguen tindre en la química medicinal. Una recopilació general dels diferents sistemes d'encapsulació existents (basats en partícules) que siguen adaptables per a l'ió de bor també està present en el treball. De la mateixa manera, els diferents mètodes que s'utilitzen per a la detecció d'ions una vegada introduïts en el cos humà i el seu seguiment són analitzats. A continuació, s'analitzaran les aplicacions i experiments duts a terme en el relacionat amb el lliurament d'ions de bor mitjançant sistemes de partícules, tant en casos ja demostrats fa temps com en els últims estudis sobre aquest tema. La finalitat d'aquest treball és recopilar la informació existent sobre les aplicacions de sistemes basats en partícules per a l'alliberament d'ions (especialment del bor) i presentar-la d'una manera que facilite la comprensió de l' importància i impacte que aquests sistemes tindran en un futur pròxim per a aplicacions biomèdiques.

Paraules clau: bor, nanopartícules, alliberament d'ions, partícules, encapsulació.

Index:

1. Introduction

In the last decades, the field of medicine has suffered a huge impact because of the advances in technology, and what we are capable of doing today was unthinkable 50 years before. Increasingly, medicine field tends to produce biomaterial nano systems for the treatment of diseases. What years ago, was to swallow a pill and wait to the drug to act, today the intake, intravenous or through oral delivery, is smart, not only reaching the target tissue but also releasing the drug in a sustained and controlled way. This covers a wide range of possibilities at the time of facing new treatments because it allows to directly reach the focus of the problem. Which has make it more and more important have been the discoveries made in cell markers (specially in cancerous cells) and the development of particle synthesis with specific properties, while at the same time the synthesis methods allows loading the desired compound in the particles for drug delivery.

In the same way the development of the mentioned cases is being huge, the same happens because new beneficial properties in elements already known can be found. Compounds which not perfectly defined properties, hide some of their properties due to the limitations of the investigation technologies existing years and decades ago. Other similar cases are the compounds that with known positive effects in health, but the mechanisms involved in those effects were unknown. New discoveries in those two aspects can make us develop new approaches to face the old existing problems. Other thing that can be done is to apply developments in one field to introduce in treatments that are being used today, if particles for drug delivery suffer huge development in the following years, they can be used for therapies where the problem is that the drug does not reach the desired tissue, or it reaches undesired tissues producing adverse side effects.

This work embrace particle-based biomaterial systems for ion release, emphasising boron as a bioactive element. Other ions or elements are going to be briefly mentioned, however, the main research applies to boron (ion or compounds) in novel applications for different therapies or investigations. Boron has received growing attention in the recent. The objective of this work is to review all characteristics of boron, used in applications, and to make a specific review about the encapsulation systems for boron ion or compounds and a specific review about the different applications that this element has in biomedical applications. Not forgetting about the general description of other applications for other ions as a bioactive element and a general description about the applications and uses of drug delivery systems, drug delivery carriers and microparticles and nanoparticles. The most important citations are present in the text, but if more information is needed, at the end of the work the consulted references are shown.

1.1. Role of ions in mammals

The essential ions in mammals are known as trace elements, which are chemical elements, that are part of the living organisms in a very little concentrations, less than 100 mg/day, they are necessary for the development and they have also biocatalytic functions, thus they are essential for life. The trace elements considered geochemical are in rocks, soil and spring, runoff, and sea waters; but following the nutritive chain, these elements are also present biogeochemically in plants, animals, and humans, fundamentally affecting its existence [1].

In the case of humans, all the trace elements have their efficiency inside defined limits or normal levels of concentration and a recommended daily supplement or dose to maintain good health. The contribution of these elements to the human body is usually made through

dietary intake, which must be regular and varied to achieve the intake of all the trace elements. With the progress of chemical-analytical techniques, the determination and localisation of the trace elements in the nature and especially in the human body that intervene in a great number of biological processes, and that affect health positively as well as negatively is now possible.

To all the trace elements, which their intake is essential with the optimal feeding doses, there are some elements that must be added, like indispensable also, the vitamins and other needed elements like mineral macronutrients: Calcium, Magnesium, Potassium, Sodium, Chlorine, Iron, Sulphur and Phosphorus. The list of essential trace elements for health until now are: Manganese, Copper, Zinc, Molybdenum, Fluor, Iodine, Chromium, Selenium, Cobalt, Boron and Lithium. But besides that, additional elements exist that are still essential candidates: Rubidium, Tin, Titanium, Vanadium, Strontium, Barium, etc. While other are only pointed as toxic elements, like: Antimony, Uranium, Arsenic, Mercury, Cadmium, Lead and Aluminium [1].

Trace elements intervene as regulators in the activation of enzymes and in other cases associated to enzymes forming compounds called metalloenzymes. Both, composed principally by proteins, have an accelerator or retardant function in the cellular reactions, for example in digestion, where trace elements act as catalysators disintegrating big molecules of food, which allow the system to digest them and transport within tissues. Nowadays, we advanced the present knowledge of functions and symptoms originated for deficit or excess of the different trace elements in humans [1], such as:

- Manganese: This element intervenes in the formation of bones and in fat metabolism. The deficit of this element can produce malformations; but and excess decreases the formation of haemoglobin and can produce neurological alterations.
- Copper: This element intervenes in haemoglobin synthesis and in nervous system development. The deficit is noticed in the form of anaemia and abnormalities in central nervous system.
- Zinc: This element has great importance in immune system, in child growth retardation and in wound cicatrisation. The Zn deficit affects all these processes and the formation of bone tissue.
- Molybdenum: This element is located in liver, skin, and kidneys, it intervenes in iron and uric acid mobilisation.
- Fluor: This element has great effect in dental caries prevention as well as in bone demineralisation, however, its deficit produces calcium elimination from bones and alterations in dental enamel.
- Iodine: This element intervenes in the formation of the thyroid hormone, that regulates the process of metabolism in the organism, between those processes it is found nervous system development, whose deficit gives rise to cretinism.
- Chromium: This element acts very efficiently in glucose metabolism; thus, it has important therapeutic effects in diabetes disease.
- Selenium: This element is very important in the antioxidant defence system; therefore, it is anticancer and anti-aging. It also intervenes in the formation of thyroid hormone and avoids formation of blood clots.
- Cobalt: This element is a constituent of vitamin B_{12}, which intervenes in nervous system and cells in general maintenance, specially of red blood cells thus this element avoids anaemia.

- Boron: This element is associated to calcium metabolism and bone formation. The deficit of this element accelerates osteoporosis.
- Lithium: This element acts similar to zinc element against some viruses like herpes, it also acts as sedative.

1.2. Ion-delivery systems for Tissue Engineering applications

1.2.1. Definition

Drug delivery systems are engineered methods or process for administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals, through controlled release of therapeutic agent or targeted delivery. Nasal and pulmonary routes of drug delivery are gaining increasing importance thanks to drug delivery systems aiming for treatment of human diseases.

While drugs have been used for long to extend lives and improve health, the way of administration of those drugs has changed a lot in the las decades, and the changes are going to be even greater in the near future.

Biomedical engineers helped a lot for comprehension of the physiological barriers that can create problems at the time of efficiently deliver drugs, like transportation in the circulatory system and the movement of drugs through tissues and cells. Another contribution has been the development of different modalities in drug delivery, techniques that had been used in the clinical practice. However, even with all the advances that have been made, a lot of drugs, including those that have been discovered thanks to the most advances strategies in molecular biology, produce unacceptable side effects. That is because the drug interacts with healthy tissues that should not be targeted.

Side effects limit the capacity of designing optimum medicaments for a lot of diseases such as cancer, infectious diseases, or neurodegenerative diseases. Drug delivery systems control the release rate of a specific drug and or area in the body where it is released. But some of them can control both features.

1.2.2. Drug targeting

Delivery of a certain drug to specific cells, tissues, or organs, aiming to isolate a selective pharmacological effect in one specific area is known as drug targeting. When injected in the blood system for example, the drugs distribute uniformly to the entire body through the blood vessels. The drug is intended to reach a specific site, but it will reach other unwanted places. That may cause side effects due to the toxicity the drug may have in other sites rather than the aimed ones. Drug targeting helps to prevent that, so the main benefits of drug targeting are not only the reduced toxicity effects due to lower doses of the drug needed in the administration but also the therapeutic effects. There is a classification of two general methods of drug targeting, which are extensively used:

- **Active targeting**: This method is about specific drug or drug carrier nano systems modifications, where an active agent provides a specific affinity to certain organs, tissues, or cells, which allows to interact and recognise them. In cancer treatments for example, the characteristics mentioned above are achieved thanks to the conjugation between active agents and nanoparticles.

Those agents provide a preferent accumulation of nanoparticles in the desired places, such as, specific molecules inside cells, intracellular organelles, tumour-bearing organs, and individual cancer cells. This type of effect is achieved thanks to specific interactions as lectin-carbohydrate, antibody-antigen, and ligand-receptor [3]. The active targeting can be subclassified into three orders:

- **First order targeting**: it is when drug carrier systems have a controlled distribution to capillary beds of certain target organs, sites, or tissues. Examples of this type of targeting includes plural cavity, compartmental targeting in lymphatic systems, joints, cerebral ventricles, peritoneal cavity, and eyes.
- **Second order targeting**: it is when instead of delivering drugs to all the cells, the drug is delivered into specific ones, for example, cancerous cells in a tumour. A good example of a second order targeting is the specific delivery to Kupffer cells, located in the liver.
- **Third order targeting**: it is an improvement of the second order targeting, not only the drug delivery is specific for certain cells, but also to specific intracellular sites inside of them. An example of third order targeting is the drug getting into the cell through endocytosis thanks to receptor-based ligand.

- **Passive targeting**: It is when due to the pharmacological or physicochemical factors of a disease, an accumulation in certain sites of the drug-carriers or drugs occur. That is the reason why, in cancer treatment, it is crucial to control specifically the surface properties and size of the nanoparticles used for the drug delivery to avoid an unwanted uptake of the reticuloendothelial system (RES) [4], to improve targeting ability and maximize circulation times. To achieve those conditions, the surface of the nanoparticle should be hydrophilic, in order to avoid the clearance by macrophages, and the particle diameter should be less than 100 nm.

1.2.3. Drug carriers

Drug carriers are elements which function is to improve selectivity, safety, and/or effectiveness in the delivery of the process of drug administration. They are mainly used for a controlled release of drugs into systemic circulation. There are different ways of doing it, one of them is the triggered release of the drug, other one is the slow and sustained release during a long period of time. The last one is usually done by diffusion, while the first one can be activated by different stimulus like temperature, activation by light or changes in pH. Another use of the drug carriers is the improvement of properties in pharmacokinetics. There are many drugs with low membrane permeability or problems dissolving in water, so the drug carriers improve the bioavailability to solve those inconveniences [6].

Drug carrier, which is also referred as drug vector, is the most important thing when talking about the correct transportation of a loaded drug. The vectors transport and retain drug while they focus on delivering it in their objectives or their surroundings. They are capable of doing all those specific functions thanks to different small structural modifications [5].

How drug carriers should be

The characteristics that drug carriers should have for a proper performance are the next ones [5,6]:

- Capacity to go through blood brain barriers.
- Surface ligands must maintain their specificity and be recognized by the target cells the carrier is aiming at.
- Biofluids, plasma and interstitial fluids cannot destabilize the complex of the drug ligand.
- Of course, the carrier must be biodegradable, non-immunogenic and non-toxic.
- The drug must be released in the target (cells, tissues or inside organs), only after the internalization and recognition.

There are a lot of types of drug carriers and depending on the drug and where it is going to be delivered (the target area) the selection is going to be different. Another thing considered is the disease itself, because how it affects to the system and because the impact the drug will have. Different types of drug carriers are modified plasma proteins, lipoproteins, liposomes, soluble polymers, monoclonal antibodies and fragments, polymeric micelles, microspheres, nanoparticles, etc.

1.2.3.1. Modified plasma proteins

This type of carrier has a considerably low molecular weight, adding that they are soluble complexes, those two characteristics makes them an option for being intelligent carriers. Are considered a suitable kind of drug delivery because it is easy to modify them by attaching different things, among which are the needed drug, sugars, peptides, or other ligand molecules [7].

1.2.3.2. Lipoproteins

Well-known high-density lipoproteins and low-density lipoproteins (HDL and LDL respectively) and other kind are considered natural targeted liposomes because they contain a lipid and an apoprotein. Lipophilic pro-drugs or lipophilic drugs can be added into the lipoproteins thanks to the lipidic core they have, which provides an adhesion without the need of a covalent bonding. Glycosylation can be made in the apolipoprotein part of this structures, as well as other targeting ligands. This capability of adding and removing glycolipids leads into new targeting parts. The behaviour this kind of particles will have *in vivo* is determined by their size and charge. Blood brain barriers will be difficult to penetrate if the particle size is large. The main research done using HDL or LDL particles are devoted to drug targeting in the liver [8].

1.2.3.3. Liposomes

One aqueous (or several) compartment surrounded by phospholipidic bilayers compound a liposome [9, 10], which are small vesicles (size range from 20 to 10. 000 nm) and artificially designed. The behaviour of the liposomes *in vivo* is strongly determined by their size of the particles, lipid composition and the electric charge. Passive drug targeting or delivery into macrophages can be easily achieved thanks to the quick capture of the macrophages of many liposomes formulations, which allows a controlled release through time of the drug to the circulatory system within the macrophages.

Adding polyethylene glycol (PEG) to the particles create the liposomes known as 'stealth' liposomes, the reason behind that name is that incorporating PEG, prevents liposome recognition from phagocytic cells. These leads to a longer time in circulation and helps to the distribution of the drug into the peripheral tissues, which are difficult to reach through the circulation system [11]. If the vascular permeability is enhanced during the inflammatory response, the local accumulation of liposomes in tissues is going to be improved despite the difficulty of liposomes for extravasation from the systemic circulation to the tissues. The creation of target sensitive liposomes or fusogenic liposomes is another approach for these carriers, because after reaching the target cells and binding to them or getting inside, the liposome will become destabilized [12].

1.2.3.4. Soluble Polymers

Soluble and synthetic polymer drug carriers have been widely researched as a versatile approach. Drugs and target moieties can be encapsulated in the carrier molecule thanks to the tailor-made conjugates, which are developed through polymer chemistry. This leads to an improvement in their bioavailability.

Properties that soluble polymers tend to avoid is the attachment of the product to the cells, too much charge or rejection to water (hydrophobicity), so at the time of designing the carriers, there are a lot of things to take into account. For example, in cancer therapy, there is a common polymer that has been widely investigated, the HMPA (N(-2-hydroxypropyl) methacrylamide). The reason why this compound is under development is the properties of its specific targeting needed in chemotherapy. There are also some cellulose derivates that show promising results in drug release [14, 15].

1.2.3.5. Monoclonal antibodies and fragments

The first-time monoclonal antibodies were developed was in 1975 by the researchers Köhler and Milstein, and since then they have been used against different diseases as antibody-based therapies. In the last twenty years, the number of studies (pre-clinical as well as clinical) that have been made in the field of monoclonal antibodies and compounds derived from them have seen a great increase. The main field that concentrate the developments done with antibodies for the recognition of antigens is the treatment for cancer, almost all the strategies are targeting this therapy. This is for the interesting applications that targeting cancerous cells have, and that is thanks to the antigens present in this type of cells, usually expressed by the cells of the tumour. Thanks to this capability, the antibody-drug conjugates (ADC) have been developed, which are a combination of monoclonal antibodies and drugs, allowing the specific delivery of the drug to the target tissue (lymphomas or tumoral cell masses) [13].

1.2.3.6. Polymeric micelles

The main property of this kind of carriers is their structure, arranged in a core-shell complex. This type of structure is given by the bilayer arrangement, which provide the particle with the shell presenting water affinity (hydrophilicity) and the core presenting water rejection (hydrophobicity). The nucleus of the polymeric micelles is formed by a polymer that is biodegradable, which allows the possibility to load a not soluble drug. Even though it is preferable to use polymers which are biodegradable, if the used polymer is not toxic for the cell and the body its capable

of excrete it through urine or other ways, there is the possibility of using a polymer non-biodegradable or with a low capacity for doing so. When in the polymeric micelle there is need to use a core that has to be soluble in water, the recommendation for the drug that is going to be loaded is to present a chemical conjugation with the core and has to be hydrophobic. One of the main reasons that affect the properties of the polymeric micelles (like the physical stability) and the release of the drug is the viscosity at the time of the formation of the particle. The shell nature is going to have important effects in the stabilization of the micelle as well as in their bio-distribution, and in the relation and interaction that those micelles are going to have with the proteins of the plasma, through the cell phagocytes in the spleen and in the liver. Polymeric micelles have been used a lot in anticancer drug delivery when targets are tumours [16, 17]

1.2.4. Microparticles and nanoparticles

The main difference between the microparticles and the nanoparticles is the size range, since both are polymeric particles. In one hand, the microparticles refers to particles with sizes between 10^{-7} and 10^{-4} meters (i.e. 0.1 to 100 microns). On the other hand, the nanoparticles size range is 10^{-9} and 10^{-7} meters (i.e. 1 to 100 nanometres). Nanoparticles can be formed by 100 atoms or one-dimensional array molecules (i.e. nanowires), two-dimensional (ultra-thin layers), or tri-dimensional (nano dust, supramolecular functional molecules). Below a mass of 20 nanograms, the classic materials like metals are transformed in a new substance specie [18]. Materials used for nanoparticles fabrication show different physical and chemical properties in comparison with the volumetric materials and even with same composition materials if they are made with microparticles [19].

The obvious difference between nanoparticles and microparticles (or bigger particles) is the relation between the number of surface atoms and the atoms inside the particles [20]. In materials formed with nanoparticles, more than a 50% of the atoms of each nanoparticle are located in the edge (surface) of the structure and not in the interior. For nanoparticles of 10 nm, this value rises to 90%, that is to say that 9 of 10 atoms are located in the surface. Due to the size, the energetic behaviour of nanoparticles is controlled by the laws of quantum mechanics.

The particular and attractive properties of nanoparticles are promising for different industries: biotechnology and medicine, electronics, optics and optoelectronics, analytics, sensors, but in the first place, the branch for new materials. For nanoparticles for biomedical applications, cosmetics or ultra-fine polishing, smart glues, functional or auto reparable lacquers, flexible ceramics, glasses with extreme properties, super-efficient catalysers, photovoltaic systems of high performance, combustion cells, batteries, electrolytic reactors, ultra-filtration membranes, sensors and nano sensors, nano lasers and quantic points, ultra-thin layers in surfaces, magnetic fluids and magnetic memories are examples of products, that went through research laboratories to applications in their way for commercial products [19, 20].

Nanotechnology embraces almost all the fields for medical applications [21]. A promising field here is the use of nanoscales, functionable vehicles for the efficient transportation of drugs through biological barriers in the action site. In the development of these carriers are included systems based in lipids, macromolecules, biopolymers or hydrogels, which are usually integrated with additional functions, like the capacity of

changing the release depending on different factors, for example, due to the pH or external magnetic fields. There are considered multiple symptoms of diseases (tumours, inflammations, skin diseases, Alzheimer's disease, etc.), ways of incorporation (skin, intestine, blood circulation) and target (brain, skin, digestive tract, other organs). It also has the application for the introduction of DNA in cells (transfection: the introduction of genetic material) for the genic therapy nanoparticles are being developed like efficient transport containers.

2. Different particle-based methods for ion release

2.1. Gold nanoparticles

Nanoparticles of noble metals, and specifically, gold nanoparticles (AuNPs), exhibits excellent, and intrinsic to its nanometric size, physical, chemical, and biological properties [24]. Moreover, gold nanoparticles can be produced in different sizes and shapes and they can be easily functionalised with a wide range of ligands (antibodies, polymers, diagnose probes, drugs, genetic material, etc.) [23]. All these properties make the gold nanoparticles an interest concept for different areas, but specially in biomedical and food sectors.

Even though the usage of gold seems to be new, it is not. The colloidal gold was used in China, 4500 years ago. The discovery of Robert Koch of the bacteriostatic effect of gold cyanide against the tuberculosis bacillus stared the usage of gold in the modern medicine being introduced in tuberculosis therapy in 1920 [28]. Nowadays, gold nanoparticles stand out specially for its phototherapeutic properties. In presence of laser light, gold nanoparticles are activated, and they release heat, being efficient in the selective treatment of tumoral cells [30]. That is why in the last years a lot of investigations have been made in the application of gold nanoparticles for early detection, diagnosis, and treatment of cancer. Besides the extraordinary potential as phototherapeutic agent, the gold nanoparticles can be used for the elaboration of nanostructured particles for the selective transport and vectorisation of drugs and therapeutic macromolecules, as well as genetic therapies (plasmid vehiculation, RNA, DNA, etc.) [26]. It is also important the usage of gold nanoparticles for the elaboration of smart transport systems that provide control, in space and time, in the delivering of the therapeutic associated compound, either by an external or internal biological impulse [27, 29].

Another important application field, as mentioned before, is the food sector, where gold nanoparticles are used as an integrated part of the polymeric compounds. These polymeric compounds (that apart from gold nanoparticles they can be formed by other ions as silver, zinc oxide or aluminium oxide nanoparticles), are used in the fabrication of containers with antimicrobial properties or to increase the resistance to abrasion of those containers [25]. Likewise, time-temperature indicators engineered with gold nanoparticles have been already elaborated [31].

2.2. Silver nanoparticles

Silver has been used since ancient times mainly for water disinfection. From the XVII century, it is described as a medicinal product with multiple purposes and from XIX century, the silver nitrate solution was introduced for using it as ocular drops in new-born babies. In the decade of 1920, the FDA (Food and Drug administration) approves solutions of ionic silver (electro colloidal) as antimicrobial agents. There are indicators that shows how silver nanoparticles are in nature and how the intended production of silver nanoparticles has been made for 100 years, being one of the most commonly used nanomaterials [33]. But nanoparticles are considered if their size is between 1 and 100 nm at least in one dimension (scale: 10^{-9}m). In accordance with size reduction, the ratio between surface and volume is considerably increased, and it leads to significant modifications in the physical, chemical, and biological properties of the silver nanoparticles. Chemically, silver (Ag) possesses an atomic number of 47. The normal oxidation state is 1+, however, it can be found also in 2+ and 3+. The diverse states of silver, either salt, nanoparticles, etc, present different properties, like nanowires and quantum dots.

Silver salts and nanoparticles are used in various applications for infection control, in forms like dressings and compresses, that contain antimicrobial surfaces with silver and silver nanoparticles. The potential repeated exposure of bacteria to low concentration of silver nanoparticles could be counterproductive, a question that has been proposed about the possibility of selecting less susceptible bacteria because there is an increase of toleration against silver. Based on the biocide effect in bacteria resistance to antibiotics it is probable that long term exposition and distribution of silver nanoparticles creates a microbial adaptative response and this risk must be evaluated. With the development of nanotechnologies and nanoparticles, new approaches are being studied, with a combination of silver nanoparticles and antibiotics, mainly against bacteria with resistance to more than three types of antibiotics (multidrug resistance). There is a discussion in the limited results for clinical applications and nanomaterial combination with antimicrobial activity or assisted antibiotics (nano biotics). It can be observed adverse effects with the combination of silver nanoparticles and antibiotics.

There is an increasing number of papers about the bactericide activity of silver nanoparticles. It is suggested that the silver nanoparticles have stronger bactericide activity than ionic silver *per se*. It is also reported that silver nanoparticles have properties against bacterial biofilms [34]. However, the bactericide activity of silver nanoparticles can be enhanced if combined with cationic polysaccharides and chitosan. The antimicrobial activity of ionic silver and silver nanoparticles depends on its bioavailability and the target microorganism.

It is generally considered that ionic silver interacts with multiple microbial targets. Its antimicrobial activity is the result of the combination and alteration of microbial proteins, with an eventual structural and metabolic disruption. One of the principal targets of ionic silver is situated in the membrane of the bacteria, where it can inhibit the driving force of protons and the electron transport for the respiratory cycle. This can cause the death of the bacteria because the alteration in the permeability of the membrane. However, the antimicrobial mechanism of silver nanoparticles is not demonstrated as well as the mechanism of ionic silver. There are different mechanisms described, some of them linked to the direct interaction with the membrane, others with the generation of ionic silver from the nanoparticle. The main antimicrobial mechanism of the silver nanoparticles is related to its ability to generate more quantity of ionic silver and increment the production of reactive oxygen species. Both of the, silver nanoparticles and ionic silver, seems to share a similar mechanism against the target membrane, even if silver nanoparticles are more effective in the range of nanograms in comparison with the micrograms range of the ionic silver. The action mechanisms are related to the size of silver nanoparticles, even though it is not clear which one is the ideal size. Silver nanoparticles smaller than 80 nm can penetrate the interior and exterior bacterial membranes while if they are smaller than 10 nm, they generate a cytoplasmatic leak because of the formation of pores in the bacterial membrane, but leaves the bacterial nucleic acid extracellular proteins intact.

2.3. Alginate nanoparticles

Alginate is an anionic polymer widely used in biomedical and pharmaceutic applications, due to its unique properties like absence of toxicity, biodegradability, availability, biocompatibility, mucoadhesive properties, absence of immunogenicity and easy to gel in presence of cations. Taken into account the properties of alginate and the process to gel or to form nanoparticles, there are nanostructured systems, like highly concentrated

emulsions of oily external phase (W/O) and aqueous external phase (O/W) and nano emulsions of oily external phase (W/O), as template for the obtention of porous materials and alginate nanoparticles, that allows the controlled incorporation and release of drugs and also usable in biomedical engineering. Using emulsions as a template, a better control in the size of particles of the formed materials can be achieved. The correct selection of oils and surfactants and emulsification by low energy methods allows the obtention of biocompatible materials by industrially scalable and sustainable methods [35].

Taking into account the reticulation of sodium alginate and calcium chloride and selecting the more appropriate mixtures to be incorporated to the different surfactant systems as aqueous component, depending on the viscosity of the mixture, provide different applications based on the achieved characteristics [35]. Some of the mixtures depending on the application are:

- In the highly concentrated emulsions used as drug release: 1% sodium alginate and 0.015% calcium chloride, due to the low viscosity of the mixture and the simplicity to incorporate in the emulsion preparation.
- In the highly concentrated emulsions used as templates to the formation of porous materials and nanoparticles: 2% sodium alginate and 0.1% calcium chloride, to obtain a high amount of reticulated alginate.
- In W/O nano-emulsions used as template for the formation of nanoparticles: 0.05-0.06% of sodium alginate and around 0.1% of calcium chloride, to avoid the precipitation of alginate due to low proportion of the aqueous component.

Different alginate nanoparticles can be obtained for controlled drug delivery and release, depending on their formation process. Sodium alginate nanoparticles can be obtained if reticulated with calcium, using as a template W/O highly concentrated emulsions, with an 83% of aqueous component and a relation between liquid paraffin and a non-ionic emulsifier of 70/30 in weight, being the aqueous component a mixture of 2% sodium alginate and 0.1% calcium chloride. After removing the aqueous component and the surfactant, the nanoparticulated material obtained is characterised through electronic microscopy, noting the isodiametric form, the aggregation of particles and great polydispersity in size, characteristic of highly concentrated emulsions that are used as a template. The preparation method, from highly concentrated emulsions helps in particle aggregation and polydispersity, obtaining a nanoparticulated material, which structure is inverse to the structure of the porous obtained material from the O/W highly concentrated emulsions. If the particles obtained from the highly concentrated emulsions are compared with the ones obtained from solution, the ones formed with a highly concentrated emulsion template have higher particle density, which can be attributed to a higher alginate concentration as well as to compartmentalised structure of highly concentrated emulsions, that allows an homogeneous distribution of alginate. Likewise, the morphology is also different, being much more rounded the nanoparticles obtained from highly concentrated emulsions [35].

However, sodium alginate nanoparticles reticulated with calcium can be also obtained from W/O nano-emulsions instead of synthetizing from highly concentrated emulsions. The W/O nano-emulsion templates of the aqueous system or mixture of surfactants and non-ionic emulsifiers (1:7 in weight) or isopropyl myristate , with an 85% of isopropyl myristate and a relation between aqueous solution and surfactant mixture of 30/70 in weight, being the aqueous component a mixture of sodium alginate to the 0.05-0.06% and calcium chloride

around 0.1%. The reticulated alginate nanoparticles obtained with this method are characterized through dynamic light scattering techniques presenting sizes below 150 nm. It has been reported a decrease in the nanoparticle size when the alginate is reticulated with calcium, when it is subdued to ultrasounds after the incorporation of the oily component. So, the alginate nanoparticles (reticulated with calcium) prepared form nano-emulsions offer great possibilities in the development of new dosage methods for active principles [35].

2.4. Mesoporous silica nanoparticles

Mesoporous silica nanoparticles are a great utility nanomaterial as they have the capacity to contain, transport and release biological active substances in a controlled way thanks to the nano valves that control the release as a reaction to certain stimulus. The mesoporous silica nanoparticles are one of the most promising materials due to its interesting properties like the high loading capacity, bioavailability, easy to produce and easy to give certain morphology, size and porous diameter to charge and release the drug. Besides, these nanoparticles present high density of silanol groups in the surface, the reactivity of this groups is suitable for the incorporation of a wide group of functional groups and specific ligands for the recognition of tumoral cells and to act on them.

This porous matrix has excellent texture and structural properties, being capable of loading high great quantity of drugs that later will be released on the target tissues in a controlled way. This material shows an excellent surface reactivity, thus, can be functionalized with different molecules of different sizes, shapes and functions with a capacity to respond to stimulus changing its properties, and as a consequence, the release of the loaded cargo will be done by de nanoparticles [37]. The mesoporous silica nanoparticles can be easily synthesised in grate scale presenting great variety of shapes and functionalised surfaces using different strategies.

The nanoparticles must have at least 10 nm of diameter, to avoid renal clearance because it will be directly eliminated from the body, however, particles bigger than 400 nm are not capable of spread through tumoral interstice with enough quantity to reach therapeutic needed doses. So, the optimal nanoparticle size to guarantee a high circulation time and increase the accumulation in tumoral interstice and its cell absorption is between 50 and 300 nm.

The mesoporous silica nanoparticles can be combined with nano valves containing magnetic particles of iron oxide and zinc, creating a new delivery system that can be activated by heath [36]. This heat can be achieved by the reaction of the nano valves to oscillating magnetic fields, this way, the nano valve will not open itself in the biological system so the complete dose of the drug will reach the target tissue avoiding adverse effects and damage in healthy tissues. This kind of nanoparticles presents a thermic stability in room temperature and starts to activate when the temperature rises because of being subdued to a magnetic field.

The incorporation of iron oxide nanocrystals with superparamagnetic properties embedded inside the mesoporous silica matrix represents a very interesting alternative design of drug nanocarriers. Its capacity to be guided magnetically by an external magnetic field, allows to concentrate the nanoparticles in the target tissue. This is due to the combination of immune stimulus effect of the temperature associated to the hyperthermia produced by the application of the oscillating magnetic field. It produces a synergic effect that could improve

notoriously the actual therapies against cancer. All this, with the possibility of being removed at the end of the therapy, offers a good alternative to significantly improve the non-invasive treatment.

When these nanoparticles are in presence of an oscillating magnetic field, they are capable of increasing the temperature of the surroundings until it reaches a hyperthermia (42-47 °C) and achieve an immune stimulator effect, provoking heating in the targeted area to enhance the local immune response and inhibit the regulation processes and growth of tumoral cells [38]. This way, immunogenic death of malign cells is promoted and a greater sensibility to chemotherapeutic effects is generated.

2.5. Chitosan nanoparticles

The initial applications of chitosan nanoparticles were focused practically on the transport and controlled delivery of therapeutic agents [40, 41, 42]. However, the current applications for the chitosan nanoparticles seems to have no limits judging by the crescent development of this systems in technological and scientific fields, especially in the biotechnological sector. Probably, this success is due to how easy is to obtain these nanoparticles with relatively simple systems. There are three main applications, which are agriculture application, water treatment and controlled drug release. The last application is the only one that is going to be explained.

Chitosan is drawing attention for the synthesis of nanoparticles capable of an “smart” drug release due to its good bio adhesive properties, bioavailability, and biodegradation, besides the broad range chemical modification reactions that makes these nanoparticles even more versatile. In that sense, there are diverse studies about specific systems preparation for the transportation and effective delivery of different therapeutic agents, as hormones, genes, proteins, etc. In the same way, there are also studies about diverse administration ways, as oral, intravenous, nasal, ocular, etc [43-48].

Even though the systems based in chitosan nanoparticles presents generally low efficiency in genetic transfection, it seems that are able to take advantage of how easily are adhered to cellular wall due to the positive superficial charges that protonation of the amino groups of the chitosan provide. This, combined with the formulation of special nanoparticles (i.e. complexes of chitosan and DNA plasmids) makes them particularly effective, showing promising transfection efficiency levels comparing with those obtained with the better-known control positive samples [49].

As far as transportation and effective liberation of protein type therapeutic agents is concerned, chitosan showed that it can provide higher stability as well as provide protection against degradation during the transit through aggressive areas. As well as promote better contact with the bio membranes, which guarantees higher bioavailability [50]. There are nano developed (for the moment only tested in fish) systems based in chitosan/tripolyphosphate for the sustained nano release of gonadotropin hormone [51]. This system produces a fertilization of 13% higher than injecting the hormone alone, this shows evidence about the protection chitosan nanoparticles provide to the hormone, which is known to have a mid-short live in the bloodstream.

2.6. Solid polymer nanoparticles

The solid polymer nanoparticles are particles of at least 1µm of diameter that are synthetised from natural or synthetic polymers. The development of solid polymer

nanoparticles has been very important in the drug release field, because they have great capability to release a broad range of compounds for the different administration routes for long periods [52]. Natural polymers (proteins or polysaccharides) have not been extensively used for their impurities, and their common need to crossover with other compounds to increase the physical stability of the system, which could detract the drug encapsulation. Thus, synthetic polymers are the most used for this field. The most used ones are the poli(lactic acid) (PLA), poli(glycolic acid) (PGA) and their copolymers, poli(lactic-co-glycolic acid) (PLGA). Besides, these polymers are known for their bioavailability and for their capability to manipulate the degradation rate, thus, drug release, by increasing the hydrophobicity or the hydrophilicity of the nanoparticle [56].

The size and superficial charge of nanoparticles is crucial for its absorption. There are different opinions about which one is the optimum size because there are different applications depending on the size. For example, if there are microparticles instead of nanoparticles, they will stay in Peyer plaques (a lymphatic tissue cumulus working in the digestion) while if the size is small enough to be a nanoparticle, it will spread systematically. This is why some of the main applications of nanoparticles are oral drug delivery and cancer treatment. The small capillaries of the body have 5-6 μm of diameter, so the size of the nanoparticles that are going to be spread through the blood circulation must be significantly smaller, without the possibility to form aggregates, in order to avoid strokes [53].

The principal characteristics of the solid polymer nanoparticles are their size, encapsulation efficiency, zeta potential (superficial charge) and drug release. Drugs can be loaded into the nanoparticles in different ways, they can be encapsulated in the polymeric matrix, (in the nanoparticle nucleus), chemically conjugated with the polymer, or adsorbed in the particle surface. The most common method in the solid polymer nanoparticles is the emulsification-evaporation, this technique gave good results encapsulating hydrophobic drugs but not so good results encapsulating hydrophilic bioactive agents. The emulsification-evaporation consists in a polymer and compound solution in an organic solvent (dichloromethane or methylene chloride). However, there are other methods as doble or multiple emulsion, diffusion through emulsification, nanoprecipitation, salting-out, and interfacial polymerization. The principal factors considered in solid polymer nanoparticles synthesis are the stabiliser/surfactant type, concentration of the stabiliser, and lyophilisation process [54].

The solid polymer nanoparticles are considered by the organism as strange hydrophobic particles, so they are quickly taken by the mononuclear phagocytic system. That is the reason why, if the nanoparticles need to last longer in the circulation, they must have a surface modification in order to avoid the phagocytosis. In the same way, after a venous administration, the hydrophobic nanoparticles are quickly removed from the systemic circulation by the mononuclear phagocytic system and they end in the spleen or in the liver. If the treatment is in one of those organs, hydrophobic nanoparticles are a good option. The commonly used polymer for the surface treatment is the polyethylene glycol (PEG), which is a hydrophilic polymer, non-ionic that present an excellent biocompatibility. The PEG molecules can be added to the nanoparticles by covalent bonds as well as adding to the mixture during the synthesis of nanoparticles, so the PEG will be adsorbed in the surface [55].

The drug release from the solid polymer nanoparticles is one of the most important characteristics of these systems. There are various factors that affect this parameter. The

nanoparticles with bigger sizes have an initial lower release than the smaller nanoparticles. Besides, the bigger the drug load, the faster and higher is the drug release rate. For example, nanoparticles containing 16.7% PLA release 90% of the drug load in 24 hours, while the nanoparticles containing 7.1% of PLA, release their loaded drug in a time above 3 weeks. A quick initial release of the drug is an indicator of a bad encapsulation, or an indicator of the drug being absorbed in the exterior of the nanoparticles [52].

3. Boron ion as a bioactive element

3.1. Essentiality

Boron is a chemical element discovered in 1808 by Gay Lussac and Thenard, it is a metalloid, with properties between metals and non-metals. Boron is an essential micronutrient for vascular plants, diatom, and some green alga species, it does not seem to be essential for fungi and bacteria (except for cyanobacteria). It seems that the requirements for Boron becomes essential in a parallel way to lignification and xylem differentiation in the vegetal kingdom.

3.1.1. Boron in plants

Boron is mainly absorbed by plants as non-dissociated boric acid H_3BO_3, fundamentally through mechanisms of mass flux (65%) and diffusion (32%). Although it seems that some extension is absorbed actively as borate anion $B(OH)_4^-$, the absorption process is initially passive (by diffusion in the free space), followed by an active absorption in the internal space. Even though this is not completely demonstrated, the active component seems to be relatively small and can depend on the variety that is been cultivated or in the present assimilable amount of Boron. The active transportation of borates in plants is mediated by the transporter discovered in *Arabidopsis thaliana* plant species. [261].

The physiological functions of Boron are not yet fully understood. Probably its role in the vegetal metabolism is the most unknown between all the essential nutrients, despite the fact that it is the micronutrient with higher molar concentrations, at least in the dicotyledons, whose boron requirements are highly superior to the monocotyledons. Boron actuates always with valence III, that is why it does not intervene in any redox process inside vegetables. It has not been found in any enzymatic systems, but it can be found as an enzymatic activity modulator [57].

A general aspect in boron deficiency is the bad development of meristematic tissues, in roots as well as in sprouts. The cells manage to separate but the process is not done correctly, so leaves present an incomplete and irregular development. In roots, boron is primarily required for cell elongation, and later for cell division. Another effect of Boron deficiency is RNA and DNA synthesis inhibition. The alteration may occur because of the essential role of Boron in the synthesis of the nitrogenous bases like Uracil. This was, the ribosomes cannot be formed and as consequence, protein synthesis is adversely affected.

Another key role of Boron is the distribution and utilization of carbohydrates inside the plant. Boron deficiency causes an anormal accumulation of sugars in tissues. It is believed that boron facilitates sugar transport through cell membrane creating a sugar-borate compound. It has been also demonstrated the direct intervention of Boron in saccharose synthesis (where the Uracil is needed) and starch.

Boron is also necessary for pectin synthesis. It can be observed that cell walls present the higher concentration of Boron (up to 50% of total amount in plants). Boron deficiency causes darkening in tissues due to an accumulation of phenolic compounds. This situation prevents the oxidation of polyphenolic compounds, which leads to lignin synthesis, thus the cell walls get weak. Accumulation of phenolic compounds causes necrosis in the tissue.

As mentioned before, Boron is needed for Uracil synthesis, and Uracil is a precursor in UDP-glucose (Uridine diphosphate glucose), which is an essential coenzyme in saccharose metabolic pathway, the principal way for sugar transport. Boron deficiency also leads to calluses formation, a compound like cellulose that can obstruct the sieve tube, affecting the transport through the phloem. Also intervenes in ATPase activity, fundamental in ionic transport processes, so, Boron plays and essential role in the transport of assimilated products.

3.1.2. Boron in industry

3.1.2.1. Glass industry

Nowadays, there are a lot of characteristics of glass that cannot be achieved without boron, so this element is extensively used in this industry. Some of the productions containing boron are for example laboratory ware, LCD (liquid crystal display) screens and resistant cookware. This kind of applications for boron containing glass (i.e. borosilicate) are achieved thanks to the specific properties, such as, chemic inertness or thermal shock resistance, that are provided by its low coefficient of thermal expansion. The boron itself is the one who gives those properties, thanks to its glassy network and the structure it gives. In the past decades, scientists have been investigating on the structure of boron in melts, borosilicates, and borate glasses.

Considering the total production of glass in the world, the boron containing glass, like borosilicate, correspond to an important part [58]. In 2009 in EU-27, the production of this types of glasses was around 3 million tonnes, that from the total production in that area, the amount represents a 10% [59]. The remarkable works of Germans Otto Schott and Ernst Abbe, as well as the British Michael Faraday in the 19th century in the glass science, leaded the first steps of implantation of boron and borosilicate glasses [60]. The use of this kind of glasses started to grow significantly thanks to the discovery of the interesting properties that boron gives to the glass. Glass making and glass itself where benefited with the addition of boron, glassmakers quickly saw that the final properties of glass improved and the process to achieve the final product was easier if it was done with boron.

There are different advantages provided by boron depending on the state of the glass; the two remarkable ones are the final “cold” glass and the industrial melting. In the first one, increased chemical durability, higher thermal conductivity, decreased thermal expansion, lower tendency to form into crystals, higher mechanical strength and upgraded optical properties like brightness, colour or transparency are achieved thanks to the presence of boron. In the second one, the enhanced properties are different, devitrification inhibition, decreased melting temperatures, reduced viscosity in the melt, increased efficiency in how raw materials refract on solution (i.e. boron can provide the ability to act as a “fluxing agent” or as a “solvent”) and a better range for working (i.e. fibres are much easier to draw) [61-64].

3.1.2.2. Agriculture industry

One of the main applications of boron in industry is in nutrition and fertilization of crops. It has an important role, especially when the development of the leaves higher is, in flowering and when the fruit is formed. The boron content in

reproductive organs (anthers, ovaries, stigma, etc) is especially high. Boron also has an important positive effect in fruit formation and seed formation process [57].

Excessively dry summer periods, continued by rainy winters or springs, are prone to manifest symptoms of boron deficiency. There are a lot of fertilizers with boron used for provide this element when it is needed: borax ($Na_2B_4O_7 \cdot 10H_20$), sodium tetraborate ($Na_2B_4O_7$ or $Na_2B_4O_7 \cdot 5H_2O$), sodium ($Na_2B_{10}O_{16} \cdot 10H_2O$), solubor ($Na_2B_8O_{13} \cdot 4H_2O$), boric acid ($H_3BO_3$), colemanite ($Ca_2B_7O_{11} \cdot 5H_2O$), etc. Sodium borates are the classic source of boron, but the application is exclusively for the soil, boric acid and solubor can also be applied in leaves with a 0.05-0.1% of boron concentration, thanks to having higher solubility and compatibility with the majority of pulverized products. The borated fertilization is frequently done by incorporation of this element to common fertilizers [57].

The applied doses can range between 0.3 and 3 kg of boron per hectare, depending on the age and sensibility of the crop, type of soil, climatology, deficiency scale, localization of the application, etc. But it must be done with care because it can easily go from deficiency situations to toxicity situations. That is why it is of extreme importance the uniformity of the application. Other important concept is that direct contact between the borated compounds and the seed must be avoided, because of risk in toxicity terms. The better time to apply boron will be determined by different factors, like local timing for rains, boron retention capacity by the ground, phenological state, crop level of deficiency, etc. [57]

3.1.2.3. Ceramic industry

Boron has been an essential ingredient in ceramic and porcelain (vitreous) enamels for centuries. Enamels are the melted glass or thin coatings applied over the surface of floor, ceramics, crockery (i.e. Chinese porcelain), and sanitary devices that provide brightness and resistance. The natural enamels are similar in nature, but they are used in metal object as pots and pans, electrical appliances, bathtubs, storage tanks and silos, architectural panels, and signboards [65].

Already before of the use of boron in glass and enamels, the borates where incorporated in ceramic frits and in production of all kind of tiles. Frits are materials of vitreous nature with a lot of silica (SiO_2), obtained by a fusion of a mixture of different crystalline materials in high temperatures (as far as 1550 °C), followed by a quick cooling of the melted mass.

In enamels, the borates are used to start the glass formation and reduce the viscosity, which helps in creating a flat surface and reducing the thermal expansion. That provides a good fitting between the varnish or the enamels and the area that coats. Boron also allows to increase the refraction rate (or brightness), improve the mechanical durability and the resistance to chemical products, as well as dissolving colorant agents.

Recently, borates have gain acceptance as an important ingredient in ceramic bodies, since it allows to the fabricants to reduce the thickness in tiles, using a wider range of clay, increase of productivity, reduced usage of energy, because of fluxing properties of boron, reducing the melting point between the vitreous matrix and the enamel.

Another use of boron in ceramics is the boron carbide, only the diamond and the cubic boron nitride are harder. This material has a high wear resistance, high compressive strength, and low density, and it is generally produced by hot press or isostatic hot press techniques to obtain a better sintering. Some of the typical applications of boron carbide in ceramics are ballistic protection, mouthpieces, and nuclear applications where neutron absorption is needed. If used as a dust, the boron carbide is excellent for grinding paste. Some of the characteristics of this boron compound are electrically insulating, high thermal conductivity, high stiffness resistance, available in a range of different purities, and excellent resistance for strong acids and alkali in high temperatures [66].

Boron nitride is other boron derivative used in ceramics. It presents a hexagonal structure and is commonly named as white graphite, due to its slipperiness, anisotropic properties, heat resistance and high thermal conductivity. This last property combined with a low thermal expansion derives in excellent thermal shock resistance. The three production methods of hot press, pyrolytic and hot isostatic press can be used in very high temperatures thanks to this compound. However, in a very oxidant atmosphere, the maximum usable temperature is 850-900 °C. Boron nitride pressed by hot press or isostatic hot press are produced through dust densification while pyrolytic boron nitride is produced by chemical deposition in vapor phase, depositing in graphite in temperatures above 1800 °C [67].

3.1.2.4. Detergent and cleaning industry

A lot of different boron compounds are used to produce laundry detergent, household or industrial cleaners and personal care products. In these applications, the unique properties of borates serve to improve elimination of stains and decolouration, enzyme stabilisation, provide an alkaline buffer, soften the water, and increase the surfactant performance. Borates also serve for bacteria and fungus control in personal care products, thanks to its fungicidal action. In soaps, borates improve notoriously ther cleaning action and reduction of levels of dirt rearrangement, leading to clean and bright clothes [68].

Some of the benefits that the boron compounds offer when are manufactured in bleaches and detergents are the next ones [70]. Perborates and borates when are in presence of calcium ions form complexes that are soluble, which helps in eliminating those calcium ions from water, softening it. Also acts as an alkaline buffer and pH controller in solutions. Enhances the performance of the surfactant. Before activating the detergent function, if the surfactants are negatively charged can interact with calcium ions and form complexes. If borates are added, not only they can form as well as negatively charged surfactants complexes with calcium ions but also, they enhanced the performance of the surfactant itself.

The boron compounds used in the detergent industry are mainly tetrahydrate perborate or sodium perborate because they have a peroxygen bond that acts in the solution as oxidizing bleaching agent deliverer. However, the sodium perborate cannot achieve the hydrolysis in any temperature, it occurs in 60 °C, except for when it is in the presence of a catalyst. Nonetheless, there are concerns due to the addition of sodium perborate to detergents, and that is because the detergents tend to end in the sewage waters, which rises the boron to enormous levels. Thus, mainly in Europe, the sodium percarbonate is being used in order to imitate the

performance of sodium perborate. That can be assumed because the sodium percarbonate can work in lower temperatures producing hydrogen peroxide, which helped in lowering the levels of boron [69, 70].

3.2. Importance of Boron in mammalian systems

3.2.1. General description

Boron acts the same way in humans and mammals. This similarity helps in obtaining trustable predictions in the effect boron will have in humans by observing the effects in lab animals, like mice. When the boron is swallowed or inhaled, it is distributed widely through all the body and something is incorporated to the bones. Then, it is quickly excreted, however, the boron incorporated to the bones lasts longer before being eliminated [71].

In laboratory animals, boron can affect the reproduction and development of the foetus and studies in rats and mice showed that boron has no carcinogenic effect. In 1994, the EPA from US classified boron as “not classifiable as to human carcinogenicity”, due to lack of data from patients and the limited quantity obtained from animals.

The few studies with humans showed that a short-term exposition to boron can cause eye irritation, upper airways, and nasopharyngeal irritation. This irritation disappears when the exposition ends. There is no evidence about long term exposition nor about fertility. However, more studies are needed to identify population groups that could be sensible to evaluate with more detail the effect boron has in reproduction.

3.2.2. Effect in reproduction and embryogenesis

It is well known that some species have the need of boron in their organisms to fulfil their life cycle [72]. The experiments with a certain frog and a certain fish provide the strongest evidences about the need of boron, because it was demonstrated how lack of boron causes atrophy in testicles and ovaries or how important boron is to determine de survival rate of foetuses [81, 81]. Sadly, the results obtained in mammals were not so definitive as the ones obtained with the specific frog for example.

However, there are results that prove the essentiality boron in the early stages of development and reproduction in the studied mammals, that mainly were rodents, pigs, and ruminants [73, 93, 91, 92]. It has been demonstrated how the development in the early stages is affected by boron deficiency as well as with boron excess. In the case of reproduction, low boron diets present less cases of implantation sites and foetus survival, more cases of dead foetuses in the uterus and less weight in the new-born as well as pig weaning weight [104, 79]. Boron presence also affects in the concentrations of Ca and P founded in the foetus and organs (ovaries, liver, uterus, etc.). In the case of early stages of development, it has been investigated how the boron plays an important role in development of immunology, embryogenesis, and psychomotor functions. It also affects the metabolism, limiting for example the intensity of lipolysis and stimulating the glucose metabolism, at the same time, it increases the concentration of certain ions in the serum, the ions mainly are Ca, P and Mg [93, 91, 92].

Plasma testosterone in rats is also affected. When boron intake is low, the testosterone concentrations are low, while when the boron intake is in the appropriate levels, testosterone concentration is higher [96]. It happens the same in human testicles when the boron intake is low. In this case of diets, the testicles (in the experiments made with

rats) suffered an atrophy but the cells responsible for testosterone production were healthy. It seems that there is not adverse effect, in terms of toxicology, when normal levels of boron are applied, the reproduction performance is correct, and no gross pathologies can be found. However, when boron levels are high, it tends to cause sterility, as well as atrophy in testicles and ovaries, it also affects the performance of ovulation and concentration of spermatozoa, which were low [75].

3.2.3. Bone growth and maintenance

Boron has effect in testosterone and oestrogen concentration in plasma, which has an impact in some elements of bone, i.e. P and Ca, and in its metabolism and usage, it also influences the bone mineralization (stimulating it). Another effect of boron in bone growth and mineralization is how the levels of proteins (specially the morphogenetic ones) seems to increase with its presence. There are natural factors that influence the level of boron in tissues (principally blood and bone tissue), for example, disease circumstances decrease boron concentration while a good health state and ageing increases it. Osteoporosis, maintenance, and mechanical properties of bone are also related to boron concentration, while low concentration leads to osteoporosis, normal concentrations enhance bone maintenance and mechanical properties [111, 73, 74, 75, 102, 82, 108, 94, 88].

Growth is also affected by boron as well as nutrient absorbability, the main reason for that is the enhanced gain ratio in digestion (feed efficiency). When dietary Ca is scarce the weight gain tends to be low, but with the presence of boron, there is increased weight gain despite of the lack of Ca. Immune organs are also influenced by boron concentration, if it is above acceptable levels, development of this organs is inhibited as well as toxicity effects. However, if the boron level is in an acceptable range, the immune organs have an increased development and growth. If the boron intake by diet is appropriate, the concentration of lipids in bones is also affected (in the case of pigs), tending to be reduced [75, 74, 90].

Boron also plays a role as an antioxidant, but it must be forming a complex with calcium. The complex CaFB has properties of an antioxidant and is called Ca-fructoborate. This property is provided by some enzymes that the boron enhances, some of those enzymes are glucose-6-phosphate dehydrogenase, glutathione peroxidase, superoxide dismutase, glutathione-S-transferase, and catalase. Another boron compound than trigger the defence mechanisms against oxidants is Boric acid or borate. Heat stress is mitigated and ROS (reactive oxygen) and RNS (nitrogen species) oxidants are detoxified by boron. Finally, peroxidation of lipids and damage taken by the DNA are weakened by boron [110, 83, 89, 78, 76, 72, 112, 88].

Another effect of the boron is in the inflammatory response because it influences the creation of the cells responsible for the inflammatory response, the macrophages. Diseases related to endotoxins have their progression supressed by borates or boric acid and CaFB, those compounds that inhibit cytokine creation as well. Leukotriene systems are decreased by boron, acting as a regulator of respiratory burst thanks to the inhibition of the compounds liberated by the white cells in the blood activated by inflammation, those compounds are serine proteases. Arachidonic acid creates prostaglandins with a pro-inflammatory activity, and the CaFB suppresses the creation of those prostaglandins. The cells have their concentration of oxidants (mainly reactive oxygen species) reduced by boron. Superoxide ions are hunted by the CaFB efficiently,

interleukin (IL)-1β, IL-6 is supressed by CaFB and nitric oxide is liberated in the culture media. There is another production that is affected by the CaFB and it is the enhancement of factor-α, which triggers tumour necrosis [84, 85, 95, 105, 106, 88].

Other property of boron is the effect it has on the aging. The principal cause of aging is when the homeostatic balance between creation and elimination of reactive oxygen and nitrogen species is disturbed, and a disproportion occurs. Aging healthier, calcification of bones and modulation of inflammatory circumstances are properties that give chances to longer life and with better quality, which are provided thanks to dietary SBE (sugar-borate esters, the most important one is the Ca-fructoborase previously mentioned). Organic boron added by diet, enhances health, specially the sugar-borate esters [88, 109].

3.2.4. Effect in hormones and enzymes

There are some substances related with essential life processes that can be influenced by boron and its dietary compounds (i.e. borates), thus the usage or metabolism of those compounds is affected by boron. Between those essential substances are included glucose, ROS and RNS, Magnesium, Nitrogen, Calcium, Copper, triglycerides, vitamin D_3, thyroid hormone, insulin, progesterone, and oestrogens [108, 72]. Some reply to hormone behaviours are affected by cell membrane functions, which are strongly related to some boron compounds as well as those compounds affect transmembrane signalling and regulation of ions through transmembrane movements. Some enzymatic systems have boron as metabolic regulator [98, 99, 107]. The composition of some tissues can be affected by boron, like bone, blood, muscles, and the central nervous system [98, 99, 100]. Other functions that are influenced by dietary boron are enzyme reactions and the ones related to cell membrane, as well as mineral and hormonal metabolism. Activity of 26 enzymes in humans and higher animals, which are essential for metabolism of energy substrate, are influenced by boron and the derivatives [84, 85, 86, 77].

Dietary boron intake is highly related to the brain and thus to behaviours. Not enough boron in the diet can produce mental fatigue and problems to stay alert or focused on complex tasks as well as the sensation of having not enough energy. The effects of low boron intake can be compared to non-specific dietary deficiency or toxicity produced by heavy metals (i.e. lead and mercury). Other behavioural aspects associated with boron deficiency are decreased vigilance and psychomotor tasks, just as supressed mental alertness, and behavioural activation [103]. On the other hand, if boron intake and concentrations instead of being low are too high, other effects may occur [111]. For example, neurological disorders and symptoms in the central nervous system like headaches, restlessness, tremors, and convulsions that can lead to death, going through weakness and coma first.

Immune system is also influenced by the presence of boron in diet [87, 101, 84, 84]. With controlled doses of an antigen, it was discovered how the production and synthesis of antibodies was enhanced by boron compounds in the humoral response. However, when the intake of boron was low, the production of IgG and IgM anti-typhoid antibodies was lower than in normal values after the injection of a vaccine containing typhoid [77]. So, the adaptative immune response is influenced by the complementation of boron in diet. The explanation for that is not totally demonstrated, but the reason

may be how some cytokines are affected by boron, which has the capability of changing the biosynthesis efficacy of those compounds [84, 113, 72].

3.3. Boron in medicinal chemistry

Boron is a well-known element that has been used for different sectors such as glass industry as borosilicate. However, the application of boron in the medicinal chemistry is gaining importance thanks to the developments made in boron chemistry [115, 117, 121, 119, 123]. Not the boron itself but its compounds are being used for drug design and determining a new class [120, 121, 122, 123]. The precursor of this class is the Bortezomib (Velcade®), it was approved in 2003 in the FDA (and in 2004 in the EU), it was the first commercialized drug containing boron for the treatment of multiple myeloma and mantle cell leukaemia for patients incapable of receiving bone marrow transplant or not responding to other treatments. Due to the success of this drug, the door for drugs with boron or boron compounds was opened, and other drugs have been approved, for example, crisaborole (Eucrisa®) and tavaborole (Kerydin®) with other applications, for onychomycosis and dermatitis (atopic and from mild to moderate) treatments respectively. So, there is an increasing number of boron compounds containing drugs that are being developed for therapeutic and a plethora of diverse applications. [114]

3.3.1. Boronic acids

Boronic acid is a derived alkyl or aril substituted of the boric acid that contains a carbon-boron link, being part of the organoboron. Boronic acids acts as Lewis acids, they are characterised by their ability to form reversible covalent complexes with sugars, amino acids, hydroxamic acids, etc. It has an acid dissociation constant around 9, but in presence of aqueous solutions, they form tetrahedral boronated complexes with values around 7. Boronic acids are commonly used in organic chemistry as chemical pieces for synthesis and as the predominant intermediate in the cross coupling of Suzuki reaction. A key concept in this chemistry is the trans metalation of its organic rests to the transition metal.

The main application of drugs based on boron compounds containing boronic acid is for cancer treatment, the main reason for that is the inhibition of proteasome caused by the boronic acid. Proteasome is a big protein complex which function is to perform the damaged or unnecessary protein degradation, it also controls de concentration of certain proteins by degradation. Proteins when are going to be degraded are marked with a protein called ubiquitin, when the enzyme ubiquitin ligase joins it, chain with affinity for ubiquitin is going to be created in the marked protein, which allows the proteasome the identification and later degradation [124, 125, 126]. The degradation managed by the proteasome is an essential mechanism in various cell processes, including the cellular cycle, the gene expression, oxidative stress, and cell apoptosis, the last one is the therapeutic target for anti-cancer activity [126].

The most common scaffold (with base of boronic acid) in drugs aiming to inhibit the proteasome is the dipeptidyl boronic acid, which is the composition of the previously mentioned bortezomib. Despite a few drugs have been proclaimed they are not commercialized yet and continue in development state [127]. Another inhibitory action that boronic acids and derivates can have if against a broad range of serine-β-lactamases [128, 129].

3.3.2. Benzoxaboroles

The main difference between benzoxaboroles and boronic acid is the properties of the oxaborole ring, particularly the boron-carbon bond, having more resistance to hydrolysis the benzoxaboroles [121]. Another important difference is the solubility in water, being also higher than in the boronic acid. Before 2006 the benzoxaboroles were used in other applications, but thanks to the anti-fungal activity discovered in that year, the compound was studied for possible therapeutic applications [116]. That is the reason why a crescent number of compounds derived from this haven been proclaimed in the last years, exhibiting plenty of bioactivities.

Between the different reported activities of the benzoxaboroles in medicinal chemistry there are studies that show an inhibition of the LeuRS (Leucyl-tRNA synthetase) in different microorganisms [130]. This synthetase is crucial in the creation of proteins inside cells, because is part of the aminoacyl-tRNA synthetase family. There are some benzoxaboroles compounds that act as anti-infection agents [135, 136]. Others act as inhibitors for synthesis of proteins of bacteria but presents toxic problems due to the inhibition of LeuRS inside cells. Because of that side effect, studies to avoid that activity were made and by adding a hydroxyl group in the molecule to the alkyl chain, the selectivity against bacterial LeuRS was higher, not only for the cytoplasmatic LeuRS but also for the mitochondrial one [131, 132]. The benzoxaboroles capability to inhibit bacterial LeuRS is due to the boron atom, which plays a fundamental performance in the mechanism of action, because it creates an adduct with the terminal nucleotide of the tRNA, heading the formation of an useless complex then producing an inhibition in protein synthesis.

Another activity of the benzoxaboroles is the inhibition of carbonic anhydrase (AC) [133]. This enzyme (which is part of the metalloenzymes family) catalyses the rapid conversion of carbon dioxide and water to bicarbonate and protons, a reaction than happens slower if the catalysator is not present. The carbonic anhydrase significantly enhances the reaction rate, and it is acknowledged as one of the reasons in the development of pathogenic microorganism, especially in their virulence and population growth. However, if the amount of carbonic anhydrase is low in humans, the appearance of various diseases may happen [134].

Anti-parasite functions can be also achieved with derivates of benzoxaboroles to different parasite strains [139]. Not only it has been used for animal ectoparasiticides [140, 141] but also for malaria [137, 138] and infectious diseases such as tuberculosis [135, 136, 147]. Potential to treat lymphatic filariasis and onchocerciasis was founded because of the properties of a benzoxaborole derivate (benzoxaborole-pleuromutilin) against the bacteria that causes those diseases [142]. There are lot of benzoxaborole derivates so are the biological activities and potential pharmaceutical applications they have, but one of the most important ones is the anti-cancer activity one of the derivates present against ovarian carcinoma, colon colorectal carcinoma and breast cancer [143]. The main effects of this derivate are the pro-apoptotic and antiproliferative performances. Attacking mainly the caspase-3 activation thus inducing a strong arrest in the cell cycle in ovarian cancer cells, thus leading the cell apoptosis and killing the dangerous cells.

3.3.3. Other

Even the main boron compounds for medicinal chemistry are the boronic acid and benzoxaborole derivatives, there are a lot of different boron compounds with pharmaceutical applications such as the boronic esters, borohydrides, diazaborines, cyclic boronic acids, boranophospates, etc. One derivative of the cyclic boronic acid was determined as option for the treatment of the neurodegenerative disease called well known as Alzheimer's disease. This compound was antioxidant and inhibitor of Aβ aggregation, being able to restrain the self-induction of Aβ aggregation [144]. Targeting the proteasome inhibition but trying to avoid the inhibition also of the serine proteases, another compound was discovered. This compound worked by producing an accumulation of p53, which causes the apoptosis of the cell [145].

Other boron compound with antibacterial activity is the peptide boronic esters. One potent inhibitor of the EcLepB (*E. coli* type 1 signal peptidase) is a compound of boronic ester-linked macrocyclic lipopeptide. Trying to develop more this compound to also show activity against other bacterial strains, another compound was discovered. This compound sacrificed a little bit the activity of the EcLepB in order to act against eight strains of bacteria [146].

When the boron atom is stabilized in the molecule with a form of heterocycle (aromatic and boron-based) the compound group is called diazaborines. The activity present in this kind of boron derivates is the inhibition of certain enzymes. One type of diazaborine was discovered as HNE (human neutrophil elastase) inhibitor [148]. This elastase has influence in distinct inflammatory diseases and it is a serine protease. Other types of diazaborines have shown different properties like good stabilization in plasma and inhibitors of different compounds as enoyl-ACP reductase [149]. This last compound is an enzyme that has an essential role in the synthesis of fatty-acids, and it is commonly present in bacteria, so if the inhibition of this enzyme is achieved, the lipid metabolism of the bacteria is going to be affected.

More compounds that are being implemented in drug design are the oxazaborines, as a boron-based approach for different applications. One of those applications was a special synthetized series made against an inflammasome (specifically the NLRP3) [150]. There are some diseases related to this inflammasome and its incorrect performance, i.e. Alzheimer's disease, gout, type II diabetes and atherosclerosis. That is because the NLPR3 inflammasome is responsible of the response of sterile inflammation and its regulation. When this inflammasome is activated, the cell mechanisms responsible of inflammatory response are triggered, and some compounds are released to do so, i.e. IL-18 and IL-1β (interleukins that are pro-inflammatory cytokines). Macrophages release the IL-1β thanks to the LRP3 (which is inflammasome dependant) and derivatives of the oxazaborines were found to inhibit those LRP3 *in vitro* as well as LPS-induced plasma *in vivo*, which increment IL-1β concentration [151, 152].

Studies about boron-pyrazole derivatives found how this type of compounds act against hepatocellular carcinoma, specifically against its proliferation [153]. It was reported that boron-pyrazole derivative acts in the interaction of cells with iron, heading the iron chelation thus interrupting the intake of boron and thus changing the regulation of iron through the signalling pathways. This action helped in achieving the apoptosis of hepatic cancer cells affecting the cell cycle and arresting the cell in the S phase. Finally, one of the last discoveries of boron compounds is the dipeptidyl boronic ester (a thioether

analogue of boron-pyrazole compound) revealed activity against triple-negative breast cancer and multiple myeloma by the hard inhibition of proteasome [154].

4. Specific particle-based methods for Boron encapsulation and release

4.1. Liposomes

4.1.1. Definition

Liposomes are the synthetic analogues of cell membranes. Its composition can be controlled in a laboratory according to its potential application. Liposomes where discovered in 1961 by Alec Douglas Bangham and Robert Home, who observed that some lipids form membranous structures when they are in contact with water [156]. In 1995, liposomes became the first approved nano vehicle for clinical use by de Food and Drug Administration (FDA) in the United States of America, whit the commercial name of Doxil, used in therapy against cancer with the active element of doxorubicin.

A liposome is a spherical structure with an aqueous centre delimited by the membrane, which is formed by a doble lipid layer. In most cases, the lipids are continued atom chains of hydrogen and carbon with a phosphate group in the border (phospholipids). The phosphate groups are hydrophilic, so they have a great affinity to water, that is why phosphate group are in the interior and exterior of the membrane. However, the carbon and hydrogen chains are hydrophobic, so they repel water and they are located between the two phosphate layers. One example of liposomes is the phosphatidylcholine, a molecule that is part of the cell membrane and it is widely used to assemble liposomes.

Liposomes are designed with a defined size in micrometric and nanometric scale. There are different methods to obtain liposomes, they are known as liposome assembly techniques Electro formation uses an electric field directed to one lipid layer to induce the grouping in structures known as giant liposomes, whose size is about 20 micron. Rehydration of lipid layer consist in adding water to a lipidic layer deposited in a recipient, which is empty of organic solvent, i.e. hexane. After that, ultrasound dispersion is made to assemble the liposomes. With this last technique micelles can be obtained, which are like liposomes but with only one lipidic layer, or multilaminar liposomes, which are liposomes with more than two layers.

Thanks to the amphipathic nature, liposomes are excellent vehicles for bioactive substances, this provide the ability to transport hydrophobic drugs in the lipidic layer as well as hydrophilic drugs in the aqueous centre or in the liposomal structure. Furthermore, the lipids in the lipidic layer of liposomes are natural components of the organism, so there is no toxic effect or incompatibility with tissues. The investigations with liposomes continue in fields like nanomedicine and pharmaceutic technology, due to its affinity with the organism (bioavailability) and degradation after administration (biodegradability) [157]. Besides, liposomes improve the therapeutic dose of the drug that reaches the target; thus, it improves their effectivity. Likewise, these lipidic nanostructures act as a vehicle for the bioactive element that provides protection against oxidation, which is essential for drugs or bioactive substances highly sensible to degradation.

4.1.2. Boron encapsulating liposomes

The first attempt to encapsulate boron in liposomes was fulfilled by [171, 172] in 1989, 1991. They were aiming at a carcinoembryonic antigen (CEA), so they conjugated a monoclonal antibody, which is specific for the CEA, with a BSH-encapsulated liposome.

Using a cell line from the pancreas and using it to immunize mice, they created monoclonal antibodies, and these new antibodies showed results of recognition of CEA. Having these antibodies against CEA, they used them to conjugate them with boron, this boron compound was in big multilamellar liposomes, so the CEA was conjugated with the liposomes, the boron compound was $Cs_2{}^{10}BSH$. The liposome where the boron compound ($Cs_2{}^{10}BSH$) was encapsulated, was produced with three different compounds in proportions of 1/1/0.05, cholesterol, egg yolk phosphatidylcholine and dipalmitoyl phosphatidyl ethanolamine, respectively. The liposomes were conjugated with the antibodies by suspension in a solution treated with them. The results *in vitro* and *in vivo* using thermal neutron irradiation for the pancreatic carcinoma cells was an inhibition of tumour growth, that was achieved because the liposomes in their surface had CEA, which provided a selective binding.

Whit the opened possibility of encapsulating boron in liposomes, new studies appeared attempting to do so. Liposomes with sizes of 70 nm or even less where synthesized in 1992 by Hawthorne and his team [162]. This boron encapsulating liposomes were made with cholesterol and DCPC (distearoylphosphatidylcholine) and encapsulating boron ions (i.e. $B_{10}H_{10}^{2-}$ and $B_{20}H_{19}^{3-}$) that were stable hydrolytically, in their form of sodium salts, that were soluble. These liposomes showed a specific delivering of the boron ions to tumours; however, these boron ions had no remarkable effect on the cancerous cells and were expulsed from the body in a quick and clean way. The results for tumour concentrations and relations between boron concentration in tumours and blood were high. Two of the boron ions showed good results interacting with different compounds inside cells, which was possible thanks to the delivering of the liposomes, that released the boron compounds directly inside the cells, preventing the contact with the blood thus the expulsion of the compound [169]. Trying to go further, another study was made with boron ions (this time produced with a reaction with ammonia) and liposomes prepared in a different way [169]. The liposomes were synthetized with PEG-200-distearoyl phosphatidylethanolamine in a concentration of 5%. These liposomes could avoid the reticuloendothelial system (RES), thus the aggregation of boron compounds in the target area was much higher (reaching 47 μg per g in boron/tumour ratio). This was thanks to the incremented lifetime of the liposome, which provided a longer circulation time of the PEGylated liposomes.

EGF-conjugated PEGylated liposomes were another approach made by the study realized by Kullberg in 2003 [164]. This time, the boron compound was soluble in water and they used different compounds. The delivery of boron showed no difference between doing it with liposome or without it in one of the compounds (boronated acridine) and it was dependant of the ligand, but the other compound (boronated phenanthridine) was not dependant of the ligand, so this last one was not a good option for future studies. Around 10^5 atoms of boron were present inside the liposomes in the first results, and after some adjustments and optimizing some processes, up to 10^6 atoms of boron can be charged inside the liposome. The experiments fulfilled with EGF-conjugated PEGylated liposomes (charged with the boronated acridine soluble in water) in living cells were made *in vitro* and with glioma cells, showing results of 6.29 ± 1.07 μg of boron per g of cells [164].

An improvement in the PEG liposomes was made, the study was aiming at a new class of liposomes that should be target-sensitive, been the target tumour tissue (from colon

tumours in mice) and aiming at reaching the interior of the cell, the improvement of the PEG liposomes was that they were transferring-coupling pendant-type [163]. One observed difference between normal cells and tumoral cells is the presence of greater amount of trans-ferrin (TF) receptors in the cancerous cells, this receptors are the responsible of introducing iron from the extracellular environment to the intracellular cytosol, and the endocytosis via these receptors is a natural physiological process. An improvement making the liposomes reach the solid tumour tissue was achieved with the TF-PEG liposomes, because having this combination provided a longer prevalence circulation time and reduced RES uptake. When this type of liposome reaches the target site (tumour cells), these cells will endocytose the liposomes mediated by the trans-ferrin. Once in the interior or the cells, the liposomes become intracellular vesicles, like endosomes. So, in tumours, the TF-PEG liposome liquidation is so damaged that they last in the interstice some time. That is why this kind of drug carriers have been studied, because they are a good approach selectively deliver ^{10}B in therapeutic concentrations to tumours [166].

4.1.3. Boron-lipid liposomes

Other approach for boron in liposomes is when boron is part of the lipid bilayer, for that, the compounds formed by boron must be lipophilic. This has two major characteristics, the increase of efficiency in boron compound embodiment and increase in boron quantity per liposome. There are two main types of liposomes containing boron in the lipidic bilayer, an hydrophilic moiety called *nido*-carborane which was developed by Hawthorne [160, 170], this kind of compound can be one tailed, as the ones developed in the first time, or two tailed [167]. The results showed that with this kind of liposomes an acceptable level of boron was achieved for the Boron neutron capture therapy (BNCT).

The synthesis process for these liposomes starts with the formation of the *nido*-carborane lipid with the boron compound, second the stability of this boron clusters is determined and then the lipids are incorporated into membranes of the liposomes [158]. The results of the study using these liposomes showed that not treated mice died in 21 days (average) of treatment while the mice that were treated with the liposomes died in 31 days (average) presenting an especial case of a mouse that survived for 52 days. However, the liposomes formed with *nido*-carborane lipids showed toxicological effects in some mice and they developed the second type of boron-lipid liposomes, the *closo*-dodecaborate lipid liposomes [168].

This type of boron lipids maintains the good characteristics of the previous ones like being an anion cluster soluble in water and divalent, while it gets rid of the toxicity effects, that is why they have been used in BNCT for clinical treatments [165]. The synthesis process of this kind of liposomes is similar to the previous ones, but with few changes, first the synthesis of the *closo*-dodecaborates is made, later these compounds are conjugated with cholesterols [161]. After that, the preparation of *closo*-dodecaborate lipid-liposomes is performed whit a fluorescent label (with PKH-67) to make easier their determination. The study used those liposomes to see the acute toxicity and accumulation in healthy mice, and they did not observe toxicity effects in normal or low boron concentrations, while if the administration was higher, toxicity was achieved, in the same way the accumulation in liver and spleen was not significant if injected liposomes were not conjugated with cholesterol [158]. However, if the

liposomes were conjugated with cholesterols, the concentrations of boron in those organs was high. The result in mice showed that the ones that were treated with these liposomes showed a significant inhibition of the tumour growth after irradiations. While in the long term (2 weeks) the tumour growth was suppressed in mice treated with this boron containing liposomes.

4.2. Chitosan nanoparticles

Aiming to prove the osteoinductive properties of boron, a polymeric scaffold for bone tissue that released particles loaded with boron was designed [173]. The boron compound used was boric acid, and the nanoparticles were of chitosan (having a mean size of 175 nm), this was achieved thanks to the ionic gelation process started by TPP (tripolyphosphate). Those boron loaded chitosan nanoparticles were later loaded into scaffolds (also made of chitosan) and firmly adhered with electrostatic interactions, through the process of freeze-dying, resulting in scaffolds presenting pores with a mean diameter of 100 µm, enough to release the nanoparticles. With the scaffolds loaded with the nanoparticles, and the nanoparticles loaded with boron (boric acid) the experiments to see the release rate and effects were performed.

The synthesis of nanoparticles is made thanks to the electric charge compounds have, being chitosan positively charged while tripolyphosphate is negatively charged, the two compounds interact with each other in the process known as ionic gelation [174]. First, a solution of acetic acid and chitosan is made, to obtain the precise concentrations. Later, the aggregation of particles needs to be avoided, for achieving so, a compound that resuspends the solution of chitosan is added (Tween 80 for example). Once the solution is ready, the boron compound that is going to be loaded in the nanoparticles is added, with an sparing agent (Mannitol for example), and when both are solved, the pH is raised (to 4.6-4.8 values). At the same time, the tripolyphosphate is added to distilled water to obtain a solution with specific concentrations. Then, the solutions are filtered, with a filter that has a membrane with pores of 0.22 µm. With the two solutions filtered, the formation of nanoparticles is made, by adding the solution of tripolyphosphate drop by drop to the other solution (the one with chitosan, mannitol and boric acid) at room temperature while it is being magnetically stirred, until the solution with 2:1 (chitosan: tripolyphosphate) volume ratio is obtained. The synthesis of boric acid loaded chitosan nanoparticles is casually performed thanks to the ionic gelation started by the tripolyphosphate.

In the study, the boric acid loaded chitosan nanoparticles were later loaded into a scaffold to be released in a controlled rate thus, the boron in cell cultures will remain longer time compared to simple boron addition to the culture medium [173]. The results of this work showed that osteogenic differentiation and proliferation of preosteoblasts was importantly affected by the delivery of boron through chitosan nanoparticles loaded in chitosan scaffolds.

4.3. Boron nitride hollow spheres

In a study aiming at a treatment for prostate cancer using boron, the delivery of boron compounds was made synthetizing hollow boron nitride nanospheres [175]. The main reason for this study was that if boron is provided in a soluble form, its life time and effectiveness is limited, and boron and its compounds have been reported to be good agents for chemotherapy [176, 177]. So, in this study, they created hollow nanospheres composed of boron nitride, with a spheroid shape and controlling its release and crystallinity to induce

the cell apoptosis of the target prostate cancer cells reducing its growth. Normally, one of the boron derivates which is sparsely soluble is the BN (boron nitride), which structure is comparable to carbon. That is the reason why this compound has been used for drug deliver (i.e. doxorubicin) as well as other compounds used for the same objective, like graphene, polymers, mesoporous silica, etc. Nonetheless, there are not so many evidence of the effects the boron nitride itself (regardless of what is carrying) has to treat cancer.

The synthesis of the boron nitride hollow nanospheres is done with the $B(OMe)_3$ (trimethoxy borane) reaction to the CVD (chemical vapour deposition), in a previously adapted method, where usually the annealing process in the second time is operated in an atmosphere full of NH_3, in this modified procedure, it is done in an atmosphere full of Ar. With the chemical vapour reaction finished, with the modified parts, the boron nitride presents a hollow nanospheres shape, low crystallinity (defined by a wide-range disorder) and a mean diameter of 200 nm. Although all the nanospheres were synthetized by the same way, some of them wile presenting the same hollow nanosphere structure, crystallinity, and approximate diameter they had differences in the wall thickness, which can vary from 20 nm to ranges of 50-60 nm.

5. Applications and experimental approaches

The particle-based methods for ion release have a lot of applications, but in this work only the ones related to the boron ion in applications for the human health are being collected. The application of boron ion for human health is relatively novel despite the fact that there are already some drugs like Bortezomib® available in the market. The main studies realised are about boron for the BNCT (boron neutron capture therapy) and about enhanced vascularization and osteogenesis. However, there is a novel approach, that considers the role of the cell membrane boron transporter (NaBC1) and its cooperation with other proteins of the cell, with a novel function for NaBC1 besides controlling boron cellular homeostasis. The realised studies about the effect of NaBC1 transporter are resulting in promising discoveries about how it works and how important boron can be.

5.1. Boron transporter (NaBC1) as a new target for biomedical applications

5.1.1. Description

The origin of this transporter is the protein BTR1, that a research team discovered to present unique transport features. They discovered that this protein in absence of borates transports OH^- (H^+) and Na^+ and in presence of borates is an electrogenic sodium (Na^+) coupled borate cotransporter, so they renamed the BTR1 protein as NaBC1 [178]. This transporter is unique for borates and it does not transport other compounds, not even the similar metalloids (like arsenate). When borates are present, it works as a voltage regulated, electrogenic cotransporter (of $Na^+/B(OH)_4^-$) presenting a stoichiometry of $Na^+/B(OH)_4^-$ [178]. The main characteristic of this ion transporter is that it can intake borates even though low concentrations and it works as a boron-concentration transporter, reinforcing the initial hypothesis describing boron as a trace element essential for life and cell homeostasis in mammals.

5.1.2. Association with other cell membrane receptors

Rico et al, have reported for the first time a novel approach using boron, but considering the NaBC1 transporter as the main target after boron activation. They are reported the interplay between NaBC1 transporter, fibronectin binding integrins ($\alpha_5\beta_1/\alpha_v\beta_3$) and grow factor receptors (GFRs) as a novel molecular mechanism not considered to date [179, 180].

The vascular endothelial grow factor (VGEF), also known as vascular permeability factor (VPF), is produced by many types of cells including tumoral cells, macrophages, platelets, keratinocytes, and mesangial cells [181]. This grow factor plays a role in normal physiological functions, like bone formation, haematopoiesis, wound healing, and human development [182, 183]. VEGF stimulates survival, proliferation, and motility of endothelial cells, initiating gemmation of new capillaries. There are three receptors with kinase intrinsic activity: VGEFR-1, VGEFR-2, and VGEFR-3, localized in endothelial or other type of cells [184]. The activation of VGEFR-1 and VGEFR-2 by the VGEF, regulates angiogenesis and vascular permeability, those two receptors possess seven domains of immunoglobulins as extracellular domains, one transmembrane region and one tyrosine kinase sequence that is interrupted by a kinase inserted in the domain [185, 186].

On the other hand, integrins are the main type of transmembrane glycoproteins that can be activated after binding to extracellular matrix proteins (ECM) such as collagen (Col), fibrinogen (FG), fibronectin (FN), laminin (LN), vitronectin (VN) or other receptors of the membrane surface such as grow factor receptors (GFR); integrins act as

mechanosensors, transmitting biochemical signals and mechanical forces, and controlling numerous cellular events [187]. There are multiple reports about the important interactions between the grow factor receptors and integrins [188, 189], and other kind of transmembrane proteins like the uPAR (urokinase plasminogen activator receptor) [190, 191].

5.1.3. Enhanced vascularization

Angiogenesis is a process that is firmly regulated by integrins, ECM (extracellular matrix) and by the dynamic interaction among grow factors (GF) [192]. Rico et al, reported a novel mechanism *in vitro* as well as *in vivo* where angiogenesis is induced with boron, with the simultaneous activation of VEGFR and NaBC1 with ultra-low doses of vascular endothelial grow factor [187]. This group present the very first predicted 3D model of NaBC1 with a sequence of proteins whit already foreseen transmembrane spans and targets for different protein kinases formerly reported [193].

This group investigated the capability of boron (in presence or absence of VGEF in physiologically ultra-low concentrations) to promote tubular-like structure organization in endothelial cells. For that, they use a PLLA (poly(L-lactic acid)) material substrates coated with fibronectin where the cells were attached. The results showed that stimulation of the NaBC1 transporter promoted the HUVEC organization while NaBC1 inhibition inhibited sprouting and organization of the same cells. They described that angiogenesis was generated after NaBC1 stimulation by enhancement of PI3k/Akt signalling pathway, involved in the effects of VEGF signalling [194]. They found that the activation of NaBC1 compensates the inhibition of PI3K/Akt signalling. Another interesting thing they discovered was that a concurrent activation of VEGF signalling and NaBC1 is not dependent to $\alpha_5\beta_1/\alpha_v\beta_3$ integrin bindings, while it induces receptor colocalization.

As conclusion to the work done by these researchers, it is reported that neovascularization with new interconnections and increased blood vessels formation, is stimulated by boron delivery from PLLA while being in an environment absent of exogenous VEGF [179]. The study exposed that boron ion release based bioengineered systems enhanced vasculogenesis trough the simultaneous activation of VEGFR/NaBC1 using vascular endothelial grow factors of the host. This novel mechanism for vascularization opens different possibilities thanks to setting a new target to investigate, which allows changes in the design (at molecular level) for biomaterials avoiding overdoses of grow factors that cause adverse effects, being able now to control much better the target receptors instead.

5.1.4. Osteogenesis induction

The multipotent mesenchymal stem cells are able to differentiate mesodermal lineages like adipogenic, chondrogenic, reticular and osteogenic. There are some conditions were usually grow factors (GFs) are included [195]. Because of that, a lot of approaches dissolve these GFs in the media they are going to use, as compounds of bioactive materials or delivered from material systems for an efficient presentation in the solid phase [196]. Nevertheless, despite being a common method in clinical trials to use these factors in high concentrations, they can produce adverse side effects such as cancer or

neurological diseases [197], so there is need a for a system that avoid the overuse of GFs.

Rico et al, proposed a novel mechanism where active NaBC1/BMPR1A and NaBC1/ $\alpha_5\beta_1/\alpha_v\beta_3$ (integrins that activate intracellular pathways) co-localization and crosstalk were involved, being the mechanosensitive ion-transporter function of NaBC1 the novel point of view, this proposed mechanism induces osteogenesis [180].They used borax (sodium tetraborate decahydrate) as source for boron ion to stimulate the NaBC1, and fibronectin (FN) to activate the FN binding integrin. The structures for the experiment where PLLA (polylactic acid, which is approved by the FDA [198]) and Glass (as control substrate), the use of PLLA was because is a biodegradable substrate for *in vivo* application in boron delivery. For the cellular system model, they used murine pluripotential C3H10T1/2 cells, which are mesenchymal cells from mouse embryo, commonly used in studies for mechanisms of differentiation, because are capable of differentiate into mesodermal lineages (chondrogenic, adipogenic, reticular and osteogenic) [199, 200].

The results of this group showed a novel approach to control the fate of mesenchymal stem cells through the interactions between specific receptors in the membrane of the cells. Their data shows a generation of an adhesion-primed state (cell spreading and mature focal adhesions), through the stimulation of mechanical determinants which intervene in the fate of the cell by actomyosin forces (actin stress fibres, pMLC), as well as the translocation of factors of transcription (YAP, pSmad) which is mechanosensitive. The stimulation (in absence of grow factors or other soluble chemicals) inhibits adipogenesis and produces enhancement of osteogenesis. This novel mechanism using boron delivery could be a great option for a controlled and safe differentiation (avoiding adverse side effects of grow factor and other soluble chemicals) of mesenchymal stem cells.

5.2. Boron neutron capture therapy (BNCT)

Boron neutron capture therapy (BNCT) is method that allows the selective destruction of tumoral cells without a significant damage of the normal tissue around. This method is based in the reactions of nuclear capture and posterior fission that happens when the ^{10}B natural and non-radioactive isotope of boron element, is irradiated with thermic neutrons of low energy (<0.4 eV) to produce its activation to ^{11}B, which decades releasing an α particle (^{4}He) and a lithium-7 (^{7}Li) nucleus. These particles, which have a high LET (Linear Energy Transfer), have a limited range in the tissue (5-9 μm), and its destructive effects are limited for the cells containing boron [202].

There are some guidelines for a correct treatment with his method, which are: boron concentration in the tumour in the irradiation moment has to be between 15 and 25 μg ^{10}B/g of tissue; the used boron compound absorption must be at least three times bigger in the tumour than in the blood and surrounding tissues; the pharmacokinetics of the compound, when it refers to boron persistence in tumour, must be compatible with the time needed to complete the irradiation; and the boron compound cannot be toxic. There are different boron compounds under research for this application, but the most commonly used are the BSH (sodium borocaptate) and BPA (boronophenylalanine) [203]. On the other hand, when it refers to neutrons, the used beam has to be appropriated. Due to the attenuation of energy the beam suffers when going through tissues, so the initial energy has to be high enough to reach the tumour with at least 0.4 eV. Because of that, in superficial

tumours a thermic neutron beam is enough, but for tumours located deep in the body, it is required an epithermal neutron beam with energies between 0.4 eV and 10 KeV [202,203].

Another aspect to consider is that the radiation field produced during the boron neutron capture therapy is really complex, with a mixture of components with high and low linear energy transfer. Besides the reaction of neutronic capture in ^{10}B (n, α) ^{7}Li, other elements present in the tissue, in important proportions, capture neutrons as well. These elements are hydrogen and nitrogen [^{1}H (n, γ) ^{2}H and ^{14}N (n, p) ^{14}C]. Besides, there are also present gamma radiation and quick neutrons in the beam, the quick neutrons collide with hydrogen nucleus and produce protons with a similar energy to the one produced by the nitrogen capture. Thus, the total physical absorbed dose will be the addition of the specific dose due to boron with all the remain contributions. Due to the neutron beam cannot be collimated because it suffers dispersion in the tissues, the neutron dose to deliver in the target, will be limited by the tolerance of the normal tissues to the unspecific dose [202, 203].

To determine the boron concentration in the organism and the eligibility of patients, it is commonly used the Positron Emission Tomography (PET). This technique is a non-invasive method that consists in the administration of a radioactive drug to observe its distribution through the organism. Commonly, in tumours, the Fluorodeoxyglucose is used despite of its low specificity, that contains ^{18}F (its main advantage is the sort life, around 110 minutes, and its innocuousness) [204], a molecule that uses the same mechanisms of transport as the glucose but cannot be metabolized. In the case of using the PET for boron neutron capture therapy, the compound must contain the ^{18}F as well as boron, which is challenged to synthetise, and the only compound that has been formed is the Boronophenylalanine.

There is a reported application of boron neutron capture therapy for treatment of undifferentiated thyroid carcinoma [201]. This type of cancer is very aggressive, with really bad prognostic and without effective treatment, and the researchers though about the boron neutron capture therapy as a viable option to treat this disease. The method consists in a selective boron absorption by the tumour and its activation through a neutron beam. The activated boron releases a nucleus of lithium-7 and one alpha particle, which have a high linear energy transfer, destroying the tumour. The human cellular line of undifferentiated thyroid carcinoma has a selective absorption of boronophenylalanine (^{10}BPA) *in vitro* as well as in NIH mice. The study proved that these animals injected with BPA and irradiated with a thermic neutron beam, a 100% control of tumour growth and a 50% of histological cure. In later works, they showed that when porphyrin $^{10}BOPP$ is injected 5 to 7 days before the BPA, the tumoral boron concentration is approximately twice the concentration obtained with the only injection of BPA (45-38 ppm vs. 20 ppm). The posterior neutron irradiation showed a 100% of complete remission in animals with tumours which pre-treatment volume was lower than 50 mm^3.

Another reported application is the optimization of boron neutron capture therapy for treating cutaneous melanoma [205]. Previously this group demonstrated *in vitro* different introduced patterns of the borated boronophenylalanine (BPA). After that, they performed biodistribution studies *in vivo* and found a positive correlation between the BPA entrapment, cell viability and tumoral temperature. Besides, they realised studies of boron neutron capture therapy in their model and checked that the measurement of tumoral temperature through thermography pre-treatment is correlated with the response to the therapy. They used mice with implanted melanoma cells, and they were irradiated. The post irradiation tumoral growth was observed for 30 days. Pre-treatment a tomography of

tumours was performed, post irradiation the tumour growth was followed and checked twice a week and once a week the tumour thermography. The short-term effect was evaluated in sacrificed animals 24 hours post irradiation, and histological studies were performed. For long term studies the tumoral growth was studied. After 30 days post irradiation it was observed that 18.75% of animals presented a total regression of the tumour (complete response) and a 43.75% showed a tumoral control in the first 20 days post irradiation (partial response), after that tumours started to grow. They concluded that the irradiation time allowed to increase in a 40% the received dose in the tumours compared with the anterior experience to the boron neutron capture therapy, increasing the dose a better response to treatment was achieved. The tumoral thermography pre-treatment allowed to identify the individual cases presented the better therapeutic success in the animal model. The tumoral thermography would be a predictive marker of the tumoral response to the boron neutron capture therapy.

6. Detection methods for Boron ion release

There are multiple methods to determine boron depending on where it is, is different to analyse a biological sample or water, in this chapter, all the known types are going to be reviewed, with special attention to the ones used in biological samples, but not forgetting about all the other methods.

6.1. Spectrophotometry

Spectrophotometry is a scientific method used to measure how much light a chemical substance absorbs, measuring the light intensity when a beam of light goes through the sample solution. This method can be also used to measure the amount of a known chemical product in a substance [206].

6.1.1. Colorimetry

Some substances develop different colours when they react with boron compounds and measuring the colour intensity, boron concentration can be determined. Some of those agents that are commonly used are azomethine-H [207], carmine [208], curcumin [209] and methylene blue [209], other ones not so popular are arsenazo, quinalizarin and crystal violet [210]. For example, if boron reacts with curcumin, different compounds can be formed depending on the medium, and are useful for boron determination. When boron react with curcumin in an oxalic acid medium rubocurcumin is formed while if the medium is sulfuric acid, rosocyanin is formed [211]. In fact, he most frequently used method with curcumin is the one that presents the reddish-brown compound of rosocyanin, because with this method 545 nm absorption can be obtained at most in pH 1.0.

However, for boron determination, the most frequently used colorimetric method is the one with azomethine-H. There are many properties this method offers in order to be one of the most popular ones, like it is easy to automatise because it does not use strong acids and it is sensitive, quick, and simple [212]. In comparison with the other methods for determination of boron in water, azomethine-H method is more sensitive and does not suffer as much interferences as curcumin and carminic acid methods [213]. Besides, better results can be obtained with azomethine-H derivates, i.e. at cost of being interfered by aluminium, copper, iron, titanium and zirconium, the azomethine-HR compound can obtain a sensitivity 3.5-fold in comparison with the azomethine-H method [214

Even though the spectrophotometric methods are widely used, they also present interferences from different elements like Aluminium, Zink, Copper, Iron and Molybdenum [215]. Another factor that affects the determination of boron is the pH of the sample, especially in the azomethine-H method (between 6.4 and 7.0 affects a lot) because of the complex formed by the boron and the azomethine-H [216]. Carminic acid method as well as azomethine-H method, present interferences (especially when the sample is a soil extract or has a high concentration of Iron) in the colour if the complex which affects the values of the spectrophotometry [217, 218]. These values are increased with iron, to avoid that, the sample is treated with thioglycolic acid, but this produces a reduction in the sensitivity, of the method [219]. Even though the spectrophotometric methods are widely used for common boron detection, they are not so good when the boron concentrations are very low. However, in 2017 a British team developed a miniaturized curcumin method for boron determination, reaching to

the limit of being able to determine 0.2 nmole of boron in a sample volume of 300 µL [220].

6.1.2.Fluorimetry

As well as boron can form compound of different colours depending on the agents it interacts with, boron can also form fluorescent complexes if mixed under milder conditions or in strong acids. The main difference between the different fluorometric methods reside in the agents that are going to react with boron. When it is used as spectrofluorimetric agent the chromotropic acid, the measurement of the wavelength of the boron complex gives a mean difference of 24 nm [221]. While when the fluorimetric agent is a sodium salt (i.e. 1,2-dihydroxyanthraquinone-3-sulfonic acid), the detection limit can be of 0.34 µg/ml [222]. It can be also used this method to detect boron and other elements (i.e. molybdenum) if instead of using only the obtained spectrum the derivatives of the first and second synchronous spectra are obtained [223]. Nevertheless, this method presents a lot of interferences from different chemical species and it is delicate with temperature or pH. Another approach is fluorimetry using carminic acid, but it has been reported to present a lot of important interferences thus is not a good option to choose [224].

6.2. Atomic spectrometry

The atomic spectrometry is an instrumental method of chemical analytics that allows to measure the specific concentrations of matter in a mixture and determines a great variety of elements.

6.2.1.Atomic emission/absorption spectrometry

These two methods are based in the atomization of the sample, by introducing the material into a flame (normally, of acetylene-N_2O-air or acetylene-air). The atomic absorption spectrometry (AAS) method works thanks to photon absorption of samples atoms in their ground state, the photons are produced with a cathode lamp loaded with the material that is going to be determined, and different amount of discrete energy is emitted. The atomic emission spectrometry (AES) on the other hand, works with the return to ground state of the excited atoms of the sample, and this method measures the energy these atoms produce when returning to the ground state. Although these methods are used for the determination of different elements, when it comes for boron, it need a special preparation of the sample, like preconcentration and separation of this element, if good results are wanted to be achieved [225].

6.2.2.Plasma source methods

The main advantage of the plasma source methods is the advantages presented in comparison with spectrophotometry, nuclear methods, and flame atomic absorption-emission spectrometry in terms of sensitivity and the ability to detect very low concentrations of boron. This is achieved thanks to the developments this kind of instruments have suffered (the plasma source analytical instruments) which provided the possibility to use plasma as ionization origin. There are different types of plasma [226], but the most important four are: glow discharge plasma (GDP) [227], direct current plasma (DCP) [228, 229], microwave induced plasma (MIP) [230] and inductively coupled plasma (ICP [231, 232,233]. The main way to produce plasma is by some gases and derivatives made by mixing them [226, 234]. Nevertheless, the one commonly used for ionization (and one of the commercial plasma-sources) is an argon inductively induced plasma. There are two main families inside plasma source methods regarding

to the instruments used for the determination, optical emission spectrometry (OES) and mass spectrometry (MS). Some examples are the inductively coupled plasma optical emission spectrometry and the microwave induced plasma mass spectrometry. The optical emission spectrometry was commonly called as atomic emission spectrometry decades ago.

The mechanism to detect boron with his method is to transform the sample into liquid, and then put into the plasma of the equipment that is going to be used for the measurement, there are different methods to introduce the sample in the plasma, and it vary depending on the objectives, reduce imperfections and to bypass sample preparation. Some of these techniques are electro thermal vaporization (ETV), laser ablation and gases [226, 234].

6.2.3. Other mass spectrometry methods

If even higher precision and accuracy are needed, as well as if the sample present isotopic composition, the thermal ionization mass spectrometry (TIMS) is one of the best options [235, 236]. When it refers to boron determination, it is used mainly for boron isotopes, especially to the ratio of B^{11}/B^{10} and in minerals [237]. The mechanism of the thermal ionization mass spectrometry is based on decomposition of the sample [238], later from the sample the elements of interest are separated and ionized [239], with the element of interest ionized [240], through a mass spectrometer the *m/z ratios* are determined [231]. There is a difference if the sample is biological or nonbiological, for the first case, the element that is going to be measured is reduced to ashes to consume the organic material while in the second case, the element that is going to be measured, is wet digested.

Another method that is based in the mass spectrometry is the spark source mass spectrometry (SSMS) and it is principally used for the measurement of trace elements concentrations of the sample [241]. Even though when isotopic samples are going to be measured the common method is the thermal ionization spectrometry, although the spark source mass spectrometry can be also used. When the determination of a small surface wants to be done, the best method is the secondary ion mass spectrometry (SIMS), method which bombards the surface with an ion beam (energetic and primary) and measures the generated ions [242]. These secondary produced ions are measured and determined in a mass analyser. The secondary ion mass spectrometry is also used in boron neutron capture therapy [243]. The reason for that is because it can measure small samples and it allows to determine boron concentration in intracellular spaces.

6.3. Nuclear reaction analysis

The nuclear reaction analysis (NRA) is a nuclear method used in material science to obtain the distribution of concentration *versus* depth for certain target chemical elements in a thin and solid film. However, for the measurement of boron is not very useful and it has only been made for academic purposes, it is based in bombing the boron atom and analysing the effect.

6.3.1. Neutron activation analysis

As the name indicates, it uses neutrons to bombard the sample that is going to be measured and it produces radioactive elements (in the sample), then, the decay of radiation in the element is measured in some cases and the radioactivity itself in other ones. One of the advantages of neutron activation analysis is that it does not destroy

the sample and it has the limit for detection commonly low as well as the ability to detect multiple elements in one solid sample. However, it cannot be used when after becoming radioactive, the sample present the possibility to have radioactive leaks in liquid volumes as well as in mass samples [244]. Another disadvantage of this method is that a nuclear reactor is needed to produce isotopes through thermally created neutron bombardment [245]. Boron does not suit in this method, because after bombarding the ^{10}B isotope with neutron it does not became radioactive thus cannot be measured with this method. It performs another reaction which produces α particles and gamma rays, which allows determination of boron by analysing the particles or the rays [243].

If the particles are analysed, the neutron activation mass spectrometry has been reported to make a simultaneous determination of boron and lithium in biological samples [246, 247], however, the sensibility of this procedure is not good enough for environmental or nutritional applications. There is also a method based in etch tracking the alpha particles, which is also known as neutron capture radiography and it is basically used to determine microscopically dissemination of boron isotopes in tissues [243]. Another technique is the neutron depth profiling (NDP), which is only used to make a surface analysis of induced positive charged particles induced with neutrons for boron determination where the isotope of ^{10}Boron is the target and alpha particles and lithium are products of the process (carried out with neutrons) [247].

Another way of determining boron from the neutron activation analysis is to focus on the gamma rays instead of focusing the alpha particles. This method is broadly used for determination of the boron isotope ^{10}B [248, 249, 250]. It works thanks to the detection of gamma rays produced in the disintegration of the nucleus after neutron bombardment [251]. Despite the sample not being destroyed the limit for detection of boron level is low. Another feature of this method is that if the amount of boron in the sample is reduced, the amount of time for a precise measurement rises logarithmically [252].

6.4. Ionometric methods

This methods usually needs a separation of boron and the matrix used as sample, and then tetrafluoroborate ion is produced (which takes few minutes), which through selective electrode for this ion it makes a potentiometric measurement, this way the concentration of boron in the sample is determined [253, 254]. However, it has been achieved a potentiometric determination without separation, although, this type of procedure is critically affected by the matrix used as sample and can suffer alterations in the potential [239, 255, 256]. To obtain sensitivity with this kind of methods, two procedures can be fulfilled, matching the calibration matrix with the one of the sample or the removal of the matrix [239]. There is a method reported to be used for boron trace amount determination in foods based in a formation of a complex between boron and beryllium and its adsorptive properties when dropped in a solution of potassium hydrogen phthalate with an electrode made of mercury, which is known as polarographic method [257]. The determination of boron obtained with this method is comparable to the results of inductively coupled plasma optical emission spectrometry. However, the ionometric method is far from being popular because other methods are better and cheaper, but there have been times where it was used in applications with borosilicate glass [258], environmental samples [259], ores, and rocks [260].

7. Conclusions

Boron is an element that is gaining a lot of importance in the field of medicine, it has been widely used in other industries for decades, but the novel discoveries of applications in the human body is changing the point of view of this metalloid. This, in addition to the gaining importance of drug delivery methods and the developing techniques for synthesis of microparticles and nanoparticles makes a huge impact in disease treatments. Boron and its compounds might be more important for the future of the human race than it was thought before.

Because of that there are three main branches, one branch that develops boron compounds to design novel drugs, exploring new effects drug complemented with boron has in our bodies. A second branch that uses the boron itself (commonly as boric acid and borax) for delivering directly in the target. Is important to mention that in this branch is where more novel discoveries are being made, thanks to the boron transporter of the cell membranes and the consequences it carries when it is inhibited or excited. Combining that to the properties of the transporter interesting discoveries are being made, like the enhancement of vascularization and osteogenesis induction, adding to that the stem cell differentiation. This kind of characteristic can be a good approach for tissue engineering as well as disease treatment. The third branch is the one that uses boron as an isotope for cancer treatment. Combining that to the properties irradiated boron has in cancerous cells is gaining a lot of importance for cancer therapy. There are promising results, but it is too soon to say that this treatment is going to defeat the cancer, maybe in some years, if the specificity of the treatment is improved and the adverse effects are reduced.

Having researched in particle-based methods for ion release, I can conclude that the microparticle methods are being overcome by the nanoparticle methods, despite the fact that the microparticles are good options in some cases, the nanometric scale is being highly developed, like nanocircuits, nanorobotics and nanomedicine. Because of that, encapsulating smaller things is being possible nowadays to release in target tissues, and that provides big steps. The reason why that is gaining more importance is because the target until now were commonly biological tissues, and sometimes cells. However, there are scientific groups today that are aiming to target the cell membrane proteins for example, a much smaller target than tumours, which demands delivery systems and ion-release systems much more precise and effective. Because of aiming more and more to smaller targets, drug delivery is so important, but it has to be considered that the loading of this kind of particles should be smaller to. That is why new approaches with elements that were thought to not have huge impact in our body are so important now, like the use of ions as a bioactive elements for biomedical and tissue engineering applications.

8. Bibliography

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