Alginate/Chitosan Microparticles for Agricultural Application

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Encapsulation into biopolymer microparticles ensures the protection and targeted delivery of active agents while offering controlled release with higher efficiency and environmental safety for ecological and sustainable plant production. Encapsulation of biological agents provides protection and increases its survivability while providing an environment safe for growth. The application of microparticles loaded with chemical and biological agents presents an innovative way to stimulate plant metabolites synthesis. This enhances plants' defense against pests and pathogens and results in the production of higher quality food (i.e., higher plant metabolites share). Ionic gelation was presented as a sustainable method in developing biopolymeric microparticles based on the next-generation biopolymers alginate and chitosan.

Keywords: ionic gelation ; sodium alginate ; chitosan ; plant secondary metabolites ; functional foods ; sustainability

1. Introduction

One of the major problems in food production is the overuse of toxic agrochemicals during the production of plants which has a serious negative impact on the environment, food safety, and consequently, on human health. Researchers are increasingly turning to natural ways of treating plants to abandon or at least limit the use of agrochemicals [1]. One of the ways to reduce their overuse is through controlled and targeted delivery of biological (i.e., microorganisms) and chemical agents. Conveniently, controlled, and targeted delivery can be achieved via the encapsulation method, and this has proven to be a suitable way of delivering nutrients for organic and sustainable crop production. Encapsulation into microparticles is an advanced, promising, and fast-developing technology that has significant advantages over other agroformulations in terms of protecting the living organisms from external conditions and the possibility of a higher survival rate. The main benefits of biological and chemical agents' encapsulation include sustained and controlled release, greater efficiency, and relatively beneficial impact on the environment [2].

As a result of consumer needs and wishes, the production of high-quality, safe, and functional food is becoming increasingly popular. The concept of functional foods refers to foods with a relatively higher content of biologically active compounds, which are thought to have a beneficial effect on human health, reduce the risk of some diseases, and they can even stimulate faster healing of tissues. Although functional foods are not fully defined, the scientific literature considers that these products provide additional benefits beyond the general benefits of nutritional intake and the pure need to satisfy hunger [3][4].

Plant secondary metabolites (PSM) are biologically active compounds often used in healthy nutrition, traditional medicine, and in a wide array of industrial applications [5][6]. Plant secondary metabolites, such as polyphenols, are composed of several groups of structurally distinct natural compounds biogenetically obtained by shikimate-phenylpropanoid-flavonoid biochemical pathways. Plants require these metabolites for pigmentation, growth, reproduction, resistance to pathogens, and many other biochemical processes and functions, while these metabolites represent adaptive characteristics that underwent natural selection during evolution. The effective defense mechanism of plants can be attributed to the wide range of PSM they synthesize [7]. With a wide range of different PSMs, plants can respond to different stressors. Given that the production of specific plant protection features can be extremely costly, new ways of enhancing defense need to be investigated and exploited. Methods involving increased expression of endogenous compounds (i.e., PSM) can significantly affect plant characteristics for resistance to invaders [7]. A high proportion of PSM can also have an important impact on human nutrition and health, by increasing the intake of antioxidants and nutrients [8]. Therefore, not only would the increase of biologically active compounds during plant cultivation have benefits for human consumption, but their primary role would be to increase the plant's defense mechanisms against pests and pathogens. Furthermore, with significant interest in the increased production of PSM, obtaining high yields can be ideal for commercial exploitation (e.g., functional ingredients). Various strategies, such as screening and selecting high-performance cell lines, cell cultures from different parts of plants, metabolic engineering, media optimization, plant growth regulators, and others, have been used so far to increase PSM production in plant cells [9], but most of these strategies are very expensive and inefficient.

Living microorganisms (e.g., nematodes, bacteria, and fungi) can be applied to the seed, surface of the plant, or soil to colonize the rhizosphere and the interior of the plant to stimulate its growth and production of PSM by increasing the supply and availability of nutrients [10]. Specifically, some studies show that mycorrhizal inoculation can increase PSM content, such as polyphenols, and increase the antioxidant activity in plants [11], but effective formulations require a carrier material that must retain mycorrhizal functional properties after the administration. One way to protect and achieve targeted delivery of microorganisms to a plant is via the encapsulation method. Microorganisms, such as the fungus of the genus *Trichoderma*, participate in the degradation of plant residues in the soil and act as biocontrol agents against plant pathogens. *Trichoderma* species synthesize specific compounds and metabolites, such as hydrolytic enzymes, plant growth promoters, antibiotics, siderophores, carbon, and nitrogen permeases. *Trichoderma* spp., among other things, stimulates plant growth by dissolving otherwise insoluble mineral nutrients, such as calcium, iron, or aluminum phosphates [12]. Strong aggressiveness against plant pathogens and high efficacy in promoting plant growth and defense mechanism have made *Trichoderma* species an important biocontrol agent [13]. However, biological agents are significantly influenced by detrimental external factors, such as pH, humidity, and ultraviolet radiation, which all impair their action. With encapsulation, a protective barrier around the biological control organism is provided, preserving its activity [14][15].

Compatibility of *Trichoderma viride* spores with divalent ions, like Cu^{2+}, Ca^{2+}, Mg^{2+} makes it pragmatic for simultaneous encapsulation into microparticles, that is, simultaneous delivery of chemical and biological agents to a plant. Calcium ions are essential macronutrients, and they have an important function in cell membrane structure and permeability, plant cell division and elongation, carbohydrate translocation, and nitrogen metabolism [16][17]. Ca^{2+} plays a regulatory role in signal transduction and absorption of nutrients through the cell membrane [17][18][19]. Ca^{2+} also signals the regulation of genes responsible for polyphenol biosynthesis [20], and binds to the phospholipid membrane, stabilizing the lipid bilayer and maintaining the structure [21]. Furthermore, it was found that in Ca^{2+}-treated plants, malondialdehyde content decreases [18][22][23]. Although the soil is known to be rich in calcium, plants often lack calcium, due to their form and relative insolubility (e.g., $CaCO_3$). In addition to Ca^{2+}, copper ions (Cu^{2+}), as well as magnesium ions (Mg^{2+}), also show a stimulating effect on PSM synthesis. They can stimulate PSM synthesis with a positive effect on alkaloid production, shikonin synthesis [24], digitalin production [25], and betaine [26]. Magnesium ion’s primary role in plants relates to photosynthesis. Mg^{2+} is an integral part of chlorophyll; it activates some enzymatic processes required for plant growth [27], participates in synthesizing DNA and RNA molecules [28], and is utilized in the plant’s cellular energy source—ATP [27]. Cu^{2+} plays key roles in photosynthetic and respiratory electron transport chains, on ethylene sensing, cell wall metabolism, oxidative stress protection, and biogenesis of the molybdenum cofactor [29]. Although Cu^{2+} is an integral part of growth media and is known to be essential for several biochemical and physiological pathways [30], it becomes relatively toxic at high concentrations [31]. Therefore, it is important to control the dose through plant growth and development to minimize excess release into the environment.

Successful delivery of the precisely controlled active substances to the right place and at the right time is a desirable characteristic of all active agent delivery systems, which may aid in precision agriculture [32]. To obtain suitable microparticles (delivery system), effective for simultaneous encapsulation of multiple active agents with appropriate controlled release, it is important to optimize the parameters during their preparation. Suitable selection of formulation variables assists in the design of microparticles with the desired release of biological and chemical for plant nutrition/protection [33][34]. This review involves the procedures of preparation and application of microparticles for the strategic delivery of biological and chemical agents, to make it available to the plant throughout its growth period. Not only to increase the proportion of PSM to protect the plant from predators and pathogens, but also, consequently, to obtain higher quality food with added value, i.e., functional food or a source of functional compounds. Also, the consumption of foods with an increased proportion of these compounds can have a beneficial effect on human health.

2. Biopolymeric Microparticles—Carrier System Composed of Alginate and Chitosan

Nowadays, research is more focused on developing natural products like, for example, natural carriers for different active agents. The most used biodegradable polymers in the process of producing microparticles are next-generation biopolymers sodium alginate and chitosan. Biodegradable polymers are used to prepare microparticles and are of main interest because they are safe for the environment and are generally non-toxic [35]. Encapsulation in biopolymeric material offers stability to the encapsulated material and can offer a controlled and targeted release of the latter. In the literature, encapsulated material is often labeled as filler, fill, core material, or internal phase, while the material used for encapsulation is called matrix, coating, shell, or external phase. Biopolymeric microparticles are usually spherical, but may have some deviations. The appearance of microparticles varies in size, shape, and composition mainly because of the influence of encapsulated material, biopolymer, and the method used for its preparation [36]. Many microparticle classifications can be found in the literature [37][38][39][40][41][42], while here in Figure 1, a representation of microparticles relevant to this review is presented wherein matrix microsphere, an encapsulated material is homogenized through the biopolymeric matrix (including surface), while shell-matrix particles combine the features of both the matrix and shell materials, where encapsulated material is not found on the particle surface, respectively.

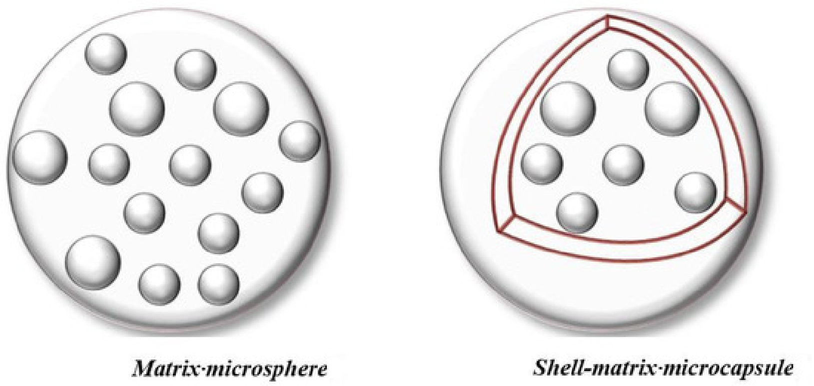

Figure 1. Representation of microparticles relevant to this review paper, a matrix microsphere, and a coated shell-matrix microcapsule.

Biopolymeric microparticles have an advantage, due to their controlled and targeted release, but some disadvantages exist, like (i) particle-particle aggregation, (ii) limitation of the storage stability, (iii) difficulty to encapsulate molecules with different degrees of hydrophilicity at the same time, (iv) challenging precise control of the dispersity of the particles. The above-mentioned disadvantages can negatively influence the efficiency of active agent delivery [43]. Even though, biopolymers are considered as great materials to use in the production of microparticles, mainly due to their abundance and affordability alongside their stability and durability throughout the process of encapsulation. Biopolymers can be easily extracted from natural sources or may be prepared with the use of microorganisms. Furthermore, biopolymers can be prepared synthetically with precision and predetermined properties as specific molecular weight, solubility, and permeability [44]. Biopolymers may vary in composition and physicochemical properties, and because of that, their utilization is often dependent exclusively on them. To achieve successful encapsulation, it is necessary to understand the structure of used biopolymers.

Sodium alginate is negatively charged, due to the presence of carboxyl groups from the uronic acid residues [45]. It is composed of two repeating carboxylated monosaccharide units (mannuronic and guluronic acids), and the ratio between them influences the properties of the biopolymer. Chitosan is a partially deacetylated polymer of N-acetylglucosamine obtained after alkaline deacetylation of chitin. The N-deacetylation is seldom complete, and the fraction of N-acetylglucosamine determines the degree of acetylation, which serves as a base to classify the biopolymer either as chitin or chitosan. When the degree of N-acetylation (defined as the average number of N-acetylglucosamine units per 100 monomers expressed as a percentage) is below 50%, chitin becomes soluble in aqueous acidic solutions (pH < 6.0) and is called chitosan [46]. The electrostatic attraction between the cationic amino groups of chitosan and the carboxylic groups of the alginate leads to the formation of the polyelectrolyte complexes of various structures. By controlling the degree of association among the functional groups, the structure and physicochemical properties of these complexes may be adjusted [47]. Immobilization and the sustained release of encapsulated agents are achievable by applying chitosan and alginate complexes in the micro/nanocapsule form [1][45][48][49].

3. A Cost-Efficient Method to Produce Microparticles—Ionic Gelation

Plenty of different techniques may be used for encapsulation in biopolymeric matrices. Hudson and Margaritis [50] comprehensively reviewed biopolymeric particles production, and divided the methods as: (i) Ionotropic gelation or external gelation, (ii) emulsification and internal gelation, (iii) the reverse microemulsion technique, (iv) emulsion crosslinking method, (v) emulsion-solvent extraction, (vi) the emulsification solvent diffusion method, (vii) emulsion-droplet coalescence method, (viii) complex coacervation, (ix) reverse micellar method, (x) self-assembly methods, (xi) water-in-oil emulsification, (xii) desolvation process, (xiii) pH coacervation method, (xiv) emulsification, (xv) nanoparticle albumin-bound (nab) technology, (xvi) self-assembly, (xvii) desolvation method, (xviii) methods involving hydrophobized pullulan derivatives, (xix) reverse micelle synthesis method, and (xx) emulsification-diafiltration. Each of the mentioned methods has its advantages and disadvantages, depending on the targeted application, but one of the most popular and widely used encapsulation methods is ionic gelation.

The ionic gelation method is often utilized in the production of biopolymeric microparticles mainly because it uses mild conditions throughout the encapsulation process and economical production costs [51][52]. Sodium alginate is a common biopolymer used to prepare biodegradable microparticles and the encapsulation procedure, and developed carrier mainly depend on its properties. Alginate-based microparticles are obtained by the dropwise addition of alginate in the bath containing divalent cations. The affinity of sodium alginate for divalent cations mainly depends on its composition. Guluronic acid-based alginate will be more prone to ion binding compared to mannuronic acid-based alginate. The affinity for the metals follows order: $Mg^{2+} < Mn^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+} < Cu^{2+} < Pb^{2+}$ [53][54][55]. These cations diffuse in sodium alginate solution forming a gel matrix, due to the cation-binding crosslinks with alginate. Crosslinking density is mainly determined as per the concentration of the cation solution [56]. The binding of the cations is related to the precise chelation process, depending on the distribution of guluronic acid blocks. This has been previously explained with the so-called “egg-box” model. The model is based on the steric configuration of the guluronic acid blocks residues [57][58]. This model describes the gel formation via sodium cation swap with calcium cation from two adjacent guluronic acid blocks and forming a single ion bridge between the chains. Ca^{2+} ions hold the alginate chains together, and with more bonds, the nature of biopolymer binds more Ca^{2+} in a stable form. Guluronic groups of alginate correctly distance coordination of Ca^{2+} between carboxyl and hydroxyl groups. This behavior is ascribed to the self-cooperative process between neighboring elements (Ising model) and is based on a physical bond with unfavorable entropy for the first divalent ion. The bond is favored for all ions to form a one-dimensional “egg-box” (a zipping mechanism) [56]. Kinetics of gelling kinetics are fast and adaptable, but also depend on polymer and cation type and their respective concentrations. With the use of a microdroplet generator (Figure 2) dripping in cation solution, microparticles can be easily prepared. A Microdroplet generator is used for the dropwise addition of sodium alginate into the bath containing divalent cations, where gelling occurs, and spheres are formed (more detail on the process can be found in Section 7.2). Conveniently, Ca^{2+} has been widely used as a gelling cation, since it is chemical versatile and safe [56][59].

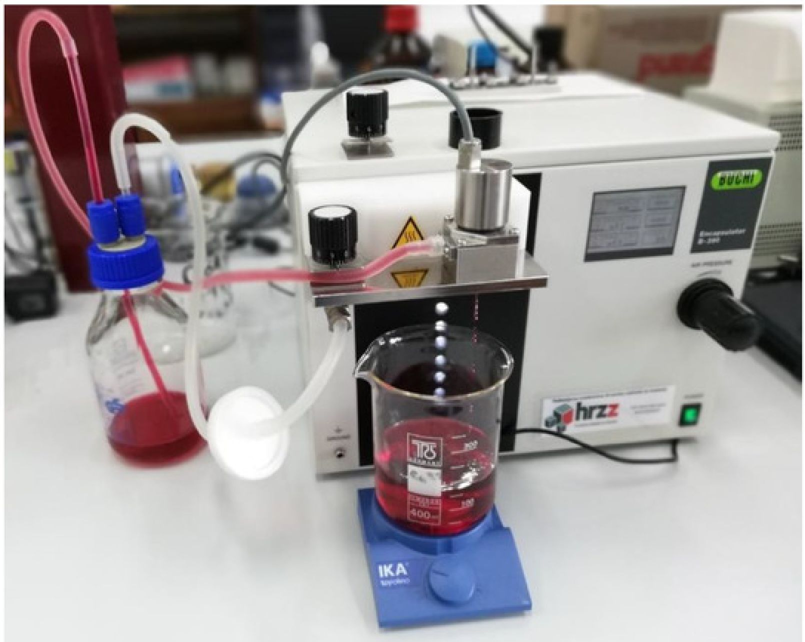

Figure 2. An example of a microdroplet generator used to prepare microparticles, Encapsulator Büchi-B390, BÜCHI Labortechnik AG, Switzerland.

Generally, sodium alginate produces rigid, but relatively porous hydrogels with weak physical and mechanical properties, which are important in delivering active agents [51][60]. Chitosan utilization is limited, due to its limited chain flexibility and poor mechanical strength, but its application has high prospects when coupled with other biopolymers [61]. Properties of these biopolymers (polyelectrolytes) may be amended by combining them, thus, improving their chemical stability and achieving microparticles with improved controlled release of encapsulated material [62]. The coating of alginate microspheres can be achieved with chitosan via polyelectrolyte complexation. The polyelectrolyte complex is dependent on the electrostatic interactions between the two oppositely charged biopolymers. It has to be noted that research on biopolymeric microparticle production is always improving and is directed in the improvement of physicochemical, functional, and release properties of used matrices keeping in mind cost-effectiveness and use of environmentally friendly or “green” material [63].

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