Chitosan-based nanoparticles for controlled release of Bromacil

By

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Abstract

Herbicides are the most widely used method for weed control. However, the agricultural industry worldwide is facing several challenges including the environmental pollution problems (soils and water) caused by the unsuitable control on the use of pesticides. Recently, nanotechnology has become an option to improve the existing pest management techniques on plants. Polymer nanoparticles can be used for storage and controlled-release of agrochemicals, such as pesticides and fertilizers. In this regard, chitosan nanoparticles have been considered for agricultural applications due to the capability of size control at the nanoscale and porosity control capability, in addition to biodegradable and biocompatible characteristics. On the above basis, this work focuses on the development of a size-controlled synthesis method for chitosan nanoparticles for further use as a controlled-release system of the herbicide Bromacil. The chitosan nanoparticles were synthesized by polymerization using methacrylic acid in water. Bromacil was added at the beginning of the synthesis process. The efficiency encapsulation of Bromacil with the nanoparticles was 62%. The release of Bromacil associated with the nanoparticle at 40 °C was 6% in 5 days. from In vitro release kinetics experiments revealed that controlled release of the herbicides from the nanoparticles, was governed by diffusion and relaxation of the polymer chains. The results indicate that chitosan nanoparticles could be used as a controlled release system of the herbicide Bromacil.

Resumen

Los Herbicidas son el método de control de malezas más utilizado. Sin embargo, mundialmente la industria agrícola se enfrenta a varios retos incluyendo problemas de contaminación ambiental (suelos y agua) causados por el uso inadecuado de plaguicidas. Recientemente, la nanotecnología se ha convertido en una opción para mejor las técnicas de control de plagas existentes en plantas. Las nanopartículas poliméricas pueden ser utilizadas para el almacén y la liberación controlada de agroquímicos como plaguicidas y fertilizantes. En este sentido, las nano partículas de chitosan se han considerado para aplicaciones agrícolas debido a su capacidad de controlar el tamaño en la escala nano, control de porosidad, en adición a sus características biodegradables y biocompatibles. En base a esto, este trabajo se enfoca en el desarrollo de una síntesis de nanopartículas de chitosan de tamaño controlado como plataforma para un sistema de liberación controlada del herbicida Bromacil. Las nanopartículas de chitosan fueron sintetizadas mediante polimerización usando ácido metacrílico en agua. El Bromacil fue añadido a la síntesis al inicio del proceso de síntesis. El % de encapsulación de las nano partículas de chitosan con Bromacil fue 62%. La liberación del Bromacil asociado con la nanoparticula a 40 °C fue 6% en 5 días. Los experimentos in vitro de la cinética de liberación del herbicida, revelaron que el mecanismo que rige la liberación es la difusión y la relajación de las cadenas poliméricas. Los resultados indican que las nanopartículas de chitosan podrían ser utilizadas como un sistema de liberación controlada para el herbicida Bromacil.

Dedicatory

To God for his blessings and the opportunity to pursue my dreams

To my husband for his unconditional love and for always believing in me

To my family for their love and support

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1. Introduction

1.1. Motivation

Weeds are one of the main limiting factors in the agricultural production [1]. Due to its ability to compete with plants for nutrients and water, among other reasons, they represent a threat to crop production. For many years, weeds were responsible of quantitative losses in food production and have impacted negatively the economy worldwide. Lately, with the growing food demand of world population this has become a matter of food security [2]. Despite the constant attempts to improve the existing pest management programs, herbicides are the most widely used method for weed control. History have demonstrated that weed control using herbicides has contributed to substantial increases in crop yields [3]. However, only a very low concentration of the herbicide actually reaches the target site of crops [4]. After the instantly liberation of the formulation active ingredient, quick losses occurred trough several degradation processes such as microbial degradation. This represent one of the main disadvantages of the conventional formulations [5]. As a result, several cycles of applications are necessary. This overuse can cause crop damage and serious environmental pollution problems because of the accumulation of the chemicals in soils and water. In addition, they can also be a risk for human health [4].

Recently, nanotechnology have been demonstrated the potential of playing an important role in several agro-industrial applications [6]. Polymer nanoparticles with diameters between 1 to 100 nm, can be used to encapsulate or adsorb an active substance. A controlled-release system of pesticides will allow the feeding of the active

ingredient at the rate required by the crop, minimizing environmental pollution and economical losses [7], [8]. The aim of a controlled release system is the slower release of the compound, enhancing the herbicide effectiveness and prolonging its effect [9]. Despite of this fact, just a few investigations have been done in the agricultural area about slow release carriers of herbicides.

The biopolymer Chitosan is receiving increased attention in the nanotechnology field because of its unique properties such as biocompability, biodegradability and non-toxicity, among others advantages [10]. Chitosan is a polysaccharide, which can be found in crustaceans as shrimps and crabs, cells walls of fungi and cuticle of insects [10], [11]. In addition, the synthesis of chitosan nanobeads shows a size control capability, which is extremely favorable in several applications in nanotechnology [12]. Chitosan has also antimicrobial properties against some fungi, bacteria, insects and viruses, which could be favorable to reduce microbial degradation of herbicides [13], [14]

The application of the herbicide Bromacil in agriculture and industrial sites is a growing practice. There's a lot of weeds (grasses and broadleaf) and plants species that can be controlled with this chemical [15]. In Puerto Rico, Bromacil is commonly used for weed control in pineapple crop [16], [17]. The herbicide effectiveness will depend largely of the application method and also that doesn't impact negatively the environment. There is a need to study these factors among others to improve the efficacy of Bromacil.

On this basis, the present work will attempt to develop a novel controlled release system of the herbicide Bromacil focuses on the size-controlled synthesis of chitosan nanoparticles, and their structural and morphological characterization.

2. Background

2.1 Weeds

Plant diseases pathogens, insects and weeds are some of the most important problems in the agricultural industry [1], [18]. All plants growing in an unwanted place are considered weeds [19]. Several organisms as fungi, bacteria, virus, nematodes and weeds, among others, can cause crop yield reductions and serious economic losses [18]**.** These yield reductions represent losses of 20 to 40% of worldwide agricultural productivity [2]. According to the Weed Science Society of America (WSSA) only in crops as corn and soybean, economic losses due weeds can be of $43 billions annually in United States and Canada [20] .

Despite plant pathogens are a serious threat to crops productivity, weeds are a major difficulty in crop cultivation systems because usually remain constant, unlike other plant pathogens that cause random outbreaks. Weeds populations always compete with crops for nutrients, water, sunlight, moisture and space [10]. Indirect damages due to uncontrolled weeds, can include a slowing in agronomic practices such as harvest and tillage [1]. Importantly, weeds have several characteristics that signify some advantages over crop plants. As example, usually weeds can grow in different environmental conditions, are self-pollinated and have an elevated rate and long stage of seed production [19]. Due to the quantitative damage caused by different species of weeds in recent decades there's has been a significant increase in the use of herbicides and pesticides in agriculture as part of pest management practices [21]**.**

2.2 Herbicides

Herbicides for weed control are one of the most widely used pesticides in the world. According to the United States Environmental Protection Agency (EPA), including all sectors (industry/commercial/government, home & garden and agriculture) in 2012, herbicides represented the largest portion usage of all pesticides with a 62% in agricultural sector (Figure 1). Applied herbicides specifically in agriculture, represents a 90% of usage in comparison with other pesticides as fungicides, insecticides, among others. In addition, herbicides world expenditures represent a 44% of total expenditures, followed by insecticides (29%), fungicides (26%) and other pesticides (1%), as nematicides and fumigants [22].

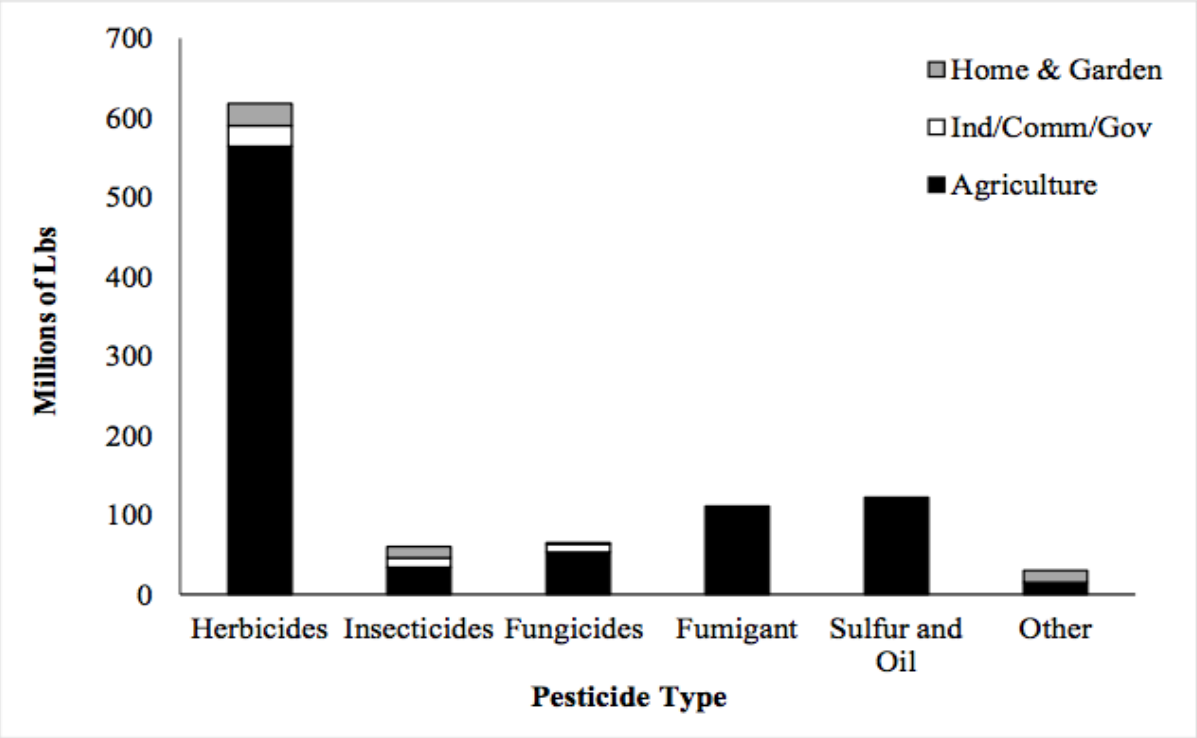

Figure 1 Conventional Pesticide Active Ingredient Usage in the United States by Pesticide Type and Market Sector, 2012 Estimates. Source: United States Environmental Protection Agency (EPA) report *[22].*

Since the introduction of herbicides in the 1940s, weed control programs have been improved successfully [19], [23]. But herbicides not only have an important role in agricultural production, they are also used in forestry and in industrial and urban sites [23].

2.2.1 Benefits of Herbicides

As mentioned before, the use of herbicides has been an extraordinary tool in agriculture and other sectors of the society [23]. Herbicides have been a fundamental element in the devolvement of crop production over the past decades. Since herbicides emerged, it has been demonstrated that farmers activities have enhanced in different ways [1]. Because infestations of weeds are controlled efficiently by herbicides, crop yields have increased. The production of some of the main crops in the world, have duplicated after the adoption of pesticides use from the decade of the 1960s to the 1990s. This not only benefit the growers who's increase their incomes substantially, it also can mean a relief for the consumer as they get lower prices for food [1]. Some findings report, that USA production of fruits and vegetables as strawberry, carrots and cotton, among others, could be reduced between a 20 to 50%, depending of the crop without the incorporation of herbicides [3].

It is public knowledge, that mechanical weed control requires a lot of hand work hours from employees and therefore this means high expenses for field owners [1]. Consequently, farm expenditures can decrease notably if the amount of human labor is reduced by the properly applied herbicides. In 2006, Earthbound Farm, one of the biggest

organic farm in United States says that “controlling weeds without herbicides takes a lot of time and is very costly” [3].

Another mechanical method to destroy weeds usually used is tillage systems [3]. Nevertheless, these procedures can generate soil erosion, which has a negative effect in agriculture productivity. These indicate that use of herbicides can reduce tillage and thereby soil erosion [1]. In other areas, herbicides formulations are widely used to controlled weeds in urban landscapes and to conserve the grass on sports parks and golf courses [23].

2.2.2 Herbicide Action

Herbicides are designed to destroy or kill weeds [24]. These chemical substances injure the plants affecting some of the main metabolic plant processes such as photosynthesis, cell division, and the synthesis of proteins, nucleic acids, lipids and pigments [19]. The series of events since the plant absorbs the herbicide until it dies is called mode of action. Site of action, is the exact site where herbicide exert its effect. The Herbicide Resistance Action Committee (HRAC) with the Weed Science Society of America (WSSA), developed an herbicide classification system according to the site of action [25]. In this way, these organizations created a uniform classification system for different countries and also help with the appropriate use of herbicides for resistance management.

Herbicides can injure certain type of weeds or plants. Herbicides that kill specific plant species are selective, whereas those that can destroy all plants are non-selective

[19]. In order to get the maximum weed control, herbicides must be absorbed into plants. Herbicides move through the plant in different ways. Systemic herbicides can translocate through the plant tissue with nutrients and water into the root system, whereas contact herbicides don't translocate and only affect the plant in the area of contact. These herbicides can be applied to weed foliage (foliar-applied herbicides) or directly to the soil (soil-applied herbicides) [26]. Soil-applied herbicides can be grouped by their timing of application as preplant, pre-emergence (PRE) or post-emergence (POST). Preplant herbicides applications have to be applied to the soil before planting the crop, whereas pre-emergence herbicides are applied after planting the crop but prior the emerging of the crop or weed. Instead, post applications are made after the emergence of the crop or weed.

One of the most important factor to consider when applying herbicides is their application method. Most of the herbicides are applied as sprays. In order to deliver to the weed the precise dose of chemical, it's necessary use the appropriate application equipment [27]**.** However, conventional pesticides applications involve an excess amount of the bioactive compound over a long period [8]**.** It is known that less than 0.1% of the herbicides applied in the field reach the target site of crops, due to problems of leaching or runoff [4]. Therefore, several cycles of addition and application become mandatory**.** Due to herbicide toxicity, excessive applications can cause crop damage, serious environmental pollution and also economic problems [8], [18].

2.2.3 Risks of herbicides

Despite the fact that pesticides are highly efficient, it is known also that they can represent a risk for human health and environment [28]**.** It has been demonstrated that some of these chemicals can have health effects in humans such as immune suppression, hormone disruption, reproductive abnormalities and cancer [23]. However, some evidence suggests that some compounds (dioxin formation) related to certain herbicides can be carcinogenic to humans. Besides during Vietnams War, veterans exposed to several herbicides formulations (2,4-D and 2,4,5T) increase the cancer risk.

In addition to those human effects, the use of herbicides can have a negative impact on the environment. Herbicides can cause water and soil contamination. Besides, due to the main purpose of the herbicides, which is injure plants, is clearly that their toxicity can reach also non-targeted vegetation and non-targeted organisms. However, all those risks should be manageable [1]**.**

2.2.4 Bromacil

Bromacil (5-Bromo-3-sec.-butyl-6-methyluracil) is an herbicide used for control of annual and perennial weeds [29]. It's applied commonly to pineapple and citrus crops and also on non-crop lands as industrial areas. Bromacil falls under the uracil chemical family, consisting of an uracil nucleus with bromine, methyl and a secondary butyl substituent (Figure 2). The commercial formula of this herbicide has bromacil lithium salt as active ingredient with the trade name of HYVAR® (Dupont), among other brands. The mode of action of Bromacil is the inhibition of photosynthesis, at the photosystem II [15]. Due the blocking of photosystem II, the electrons transport is interrupted thereby a series of reactions occur resulting in chlorosis and necrosis of the leaves and eventually the plant death. The chemical is mainly absorbed through the roots and translocated by the xylem of the plant, but also can be absorbed by the leaves and stem. The performance of Bromacil have been effective because it's toxicity is extended to many plant species. However, this herbicide has moderate adsorption in soil therefore it moves through soil and cause some environmental contamination. Groundwater can be contaminated as a result of the leaching of the herbicide through the soil.

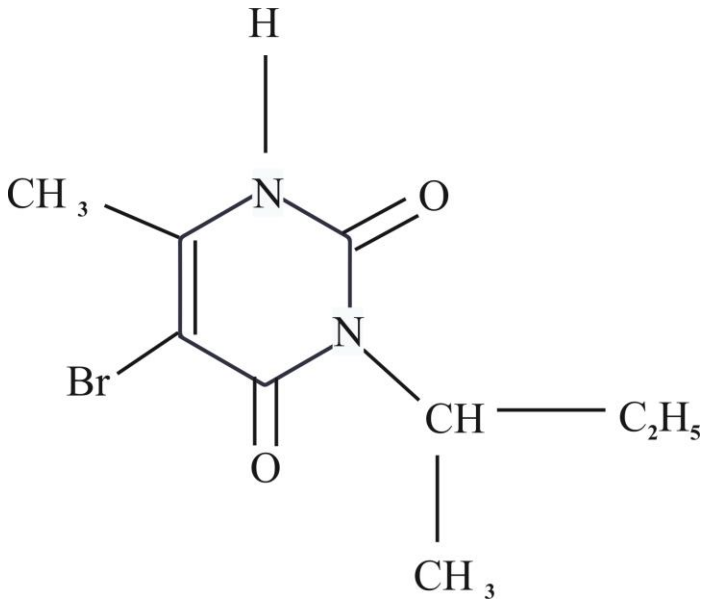

Figure 2 Chemical structure of Bromacil molecule.

2.3 Nanotechnology and Herbicide Controlled Release

Based on the above considerations, there is a strong need to find alternatives to deal with chemical losses due to degradation processes and with contamination problems. Nanotechnology have been widely used for several applications in medicine, material science, electronics, environmental remediation, energy, among others [30]**.** The term 'Nano' is a unit prefix meaning "one billionth", referring one nanometer being equal to one billionth of a meter. Through the nanotechnology field, researchers manipulate and control matter at atomic and molecular level (usually at a scale of 1 to 100nm) to design materials with novel properties [31] .

One of the advantages of the creation of systems at the nanometer scale, is the increase of surface area per mass of a material [32]. With an expanded surface area of the material, the quantity of material that can interact with close by materials will be higher.

Because of atoms on material surface are frequently more reactive than those on the center, then the material will be more reactive. An image of this phenomenon can be seemed at Figure 3.

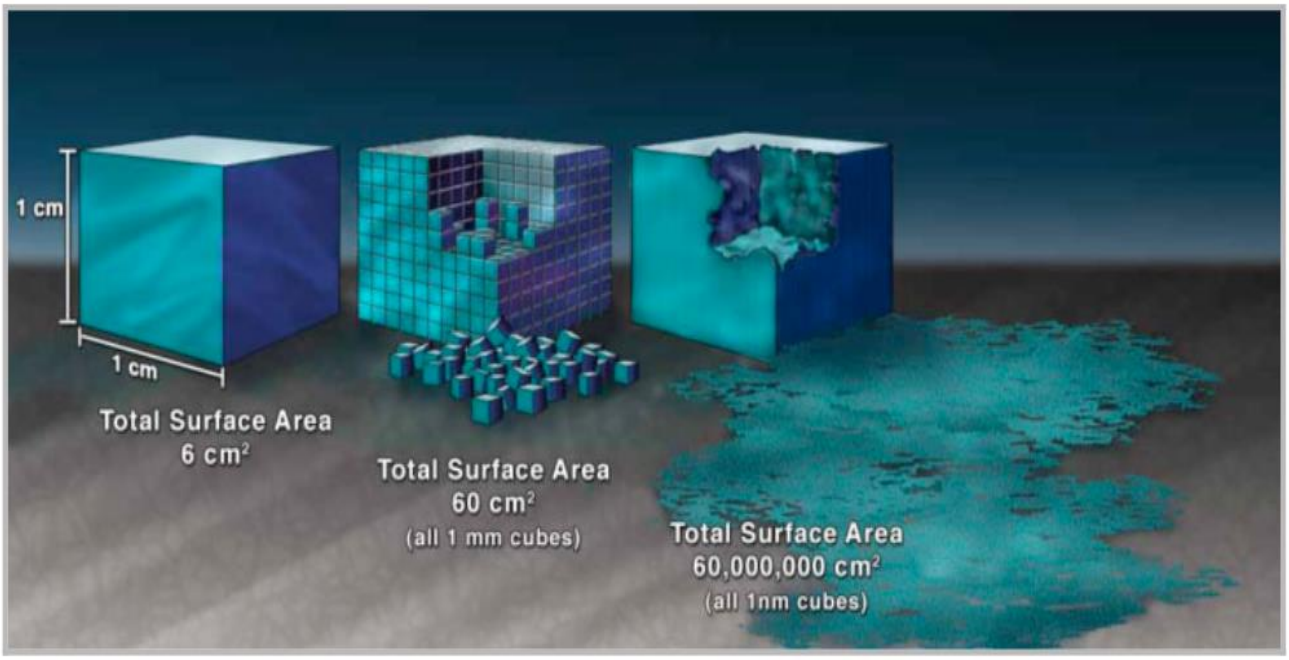

Figure 3 Effect of the increase surface area [30].

Besides, nanoscale materials present unique chemical, physical, mechanical and optical properties that may be different from properties of a larger scale material.

2.3.1 Nanotechnology in Agriculture

Recently, nanoscale science has achieved enormous attention as a potential tool in the agricultural sector [6]. Plant diseases, climate change, and environmental problems such as run-off and accumulation of agrochemicals as consequence of its excessive usage, are some of the issues that are confronting the agricultural sector worldwide [7] . In addition, as the world population increases is strongly needed the production of more healthy food [33]. Nanotechnology systems can propose potential solutions to some of those challenges in the agricultural and food industry.

In recent decades, nanotechnology have been progressing in several areas of agricultural sector. As example, researchers have been developing alternatives for liquid retention in soils, water purification and pollutant remediation. Plant genetic transformation have been also improved through nanoparticles carrying DNA to transport to plants [34]. Another novel system developed by nanotechnology are the nano-sensors. These sensors permit to detect not only pest and microbes, it also identifies nutrients and plan stress caused by temperature, presence of pathogens or nutrient deficiency. Thereby, farmers can take quick control measures and apply chemicals properly [33]. Besides, several investigations shown that nanosensors also can be used to detect pesticide residues [33], [34].

Many applications of this new technology are focused on crop protection. The development of nanomaterials can enhance plant disease management and also allow to detect pathogens faster. Furthermore, nanotechnology can be used to improve plants capacity to absorb a substance as pesticides or fertilizer resulting in a substantial increase of crop yields. This suitable absorption of the plant may be possible using a nanoparticle

with a pesticide or any substance developed to protect the crop from pathogens. On the other hand, if the active ingredient of an herbicide is integrated with a nanosystem it can eliminate weeds without environmental pollution and it can also reduce the application cycles of the herbicides [33]–[35]. This can be achieved through controlled release systems or delivery systems. There are countless applications of nanotechnology in agriculture, but some of those applications are exclusively in plant protection and nutrition and are presented in Figure 4 [35] .

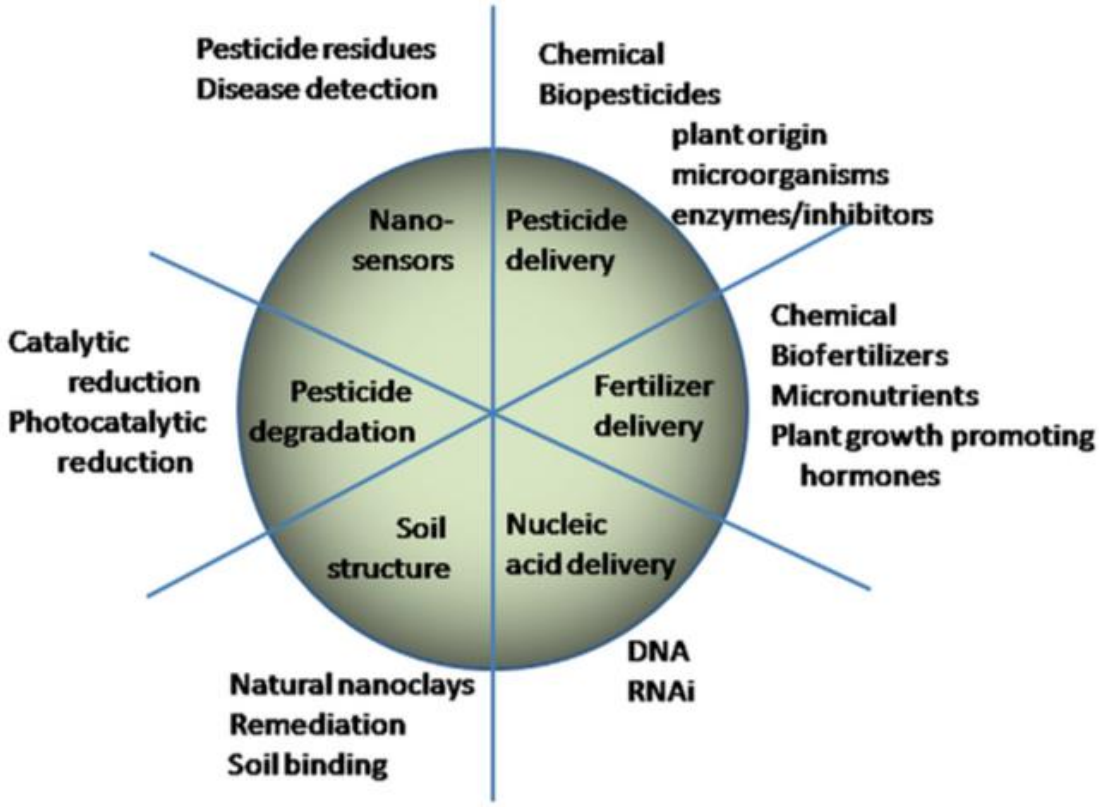

Figure 4 Applications of Nano-biotechnology in Plant Protection and Nutrition [35].

2.3.2 Controlled release system

Pesticides applications are frequently use in agricultural fields in order to control plant diseases and weeds. Pesticides application mode, soil pH and environmental conditions can influence in the loss of pesticides active agents [36]. However, on conventional pesticide applications a large quantity of the product is lost principally due the quick release of the chemical active ingredient [5]. The loss of these compounds in the field can occur by evaporation, leaching, volatilization and various degradation processes (Figure 5). Controlled release system in agriculture have been developed in order to improve the performance of conventional applications releasing the formulation slowly.

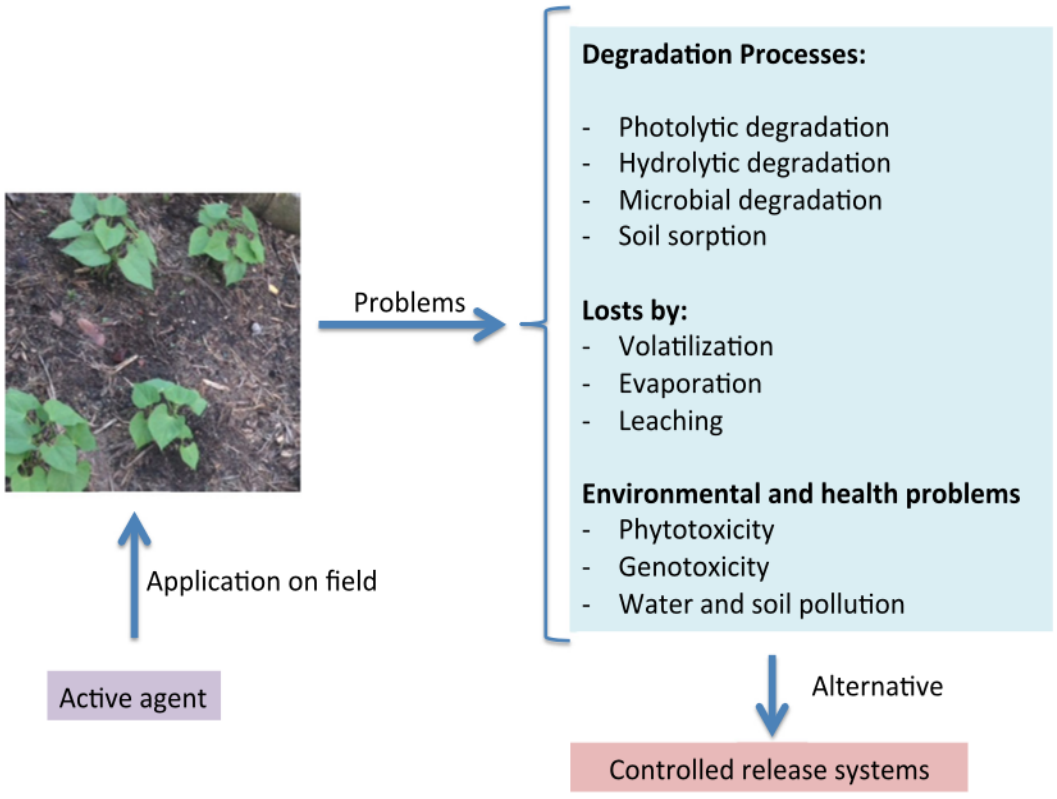

Figure 5 Schematic representation of the use of pesticides on the field and its consequences in the environment [5]

According to A. Roy *et al* [9], controlled release is “the permeation-regulated transfer of an active ingredient from a reservoir to a targeted surface to maintain a predetermined concentration level for a specified period of time”. As can be seen in Figure 6, the initial pesticide concentration in conventional applications is high and decrease abruptly staying beneath the minimum effective concentration. Instead, by using controlled release systems pesticide concentration can stay in the effective level for a longer period. Therefore, the efficiency of the pesticide will increase, avoiding excessive applications and reducing the amount of chemical supplied. Consequently, there will be a reduction of energy costs, improving the profits. At the same time, these systems will stored, protect and allow the safe handling of the active compound, keeping the field workers safe [8]**.** Some other important advantages of these techniques, are the reduction of lost by evaporation, decrease of phytotoxicity and thereby reducing environmental pollution [5], [9], [36].

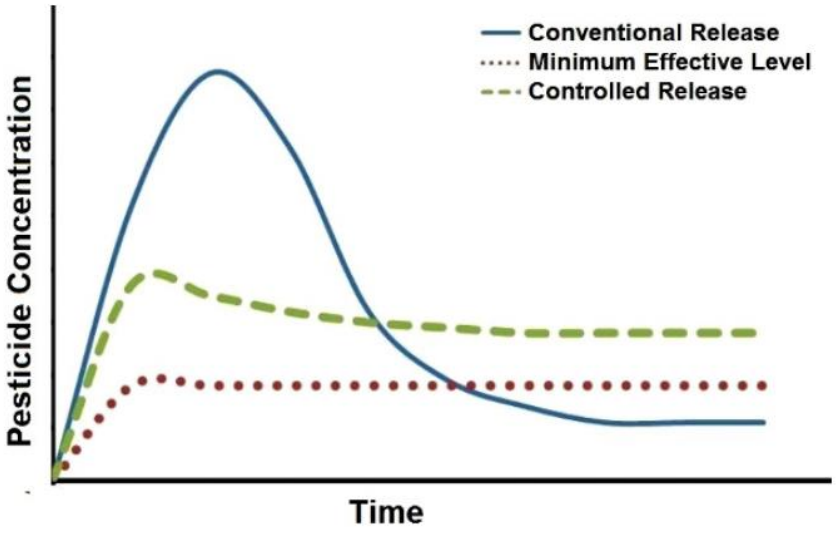

Figure 6 “Theoretical pesticide active site concentrations from conventional and controlled release” [9]

Many materials as polymers, silica, clays, polyphosphates, among others have been investigated for develop controlled release systems [5]. Among those materials, natural polymers are highly used due its unique characteristics as biodegradability, environmentally friendly and cost effectiveness. Polymers can leave minimum environmental residue, making these materials ideals for these types of systems. As well, certain properties of the polymer can be controlled to obtain the results desired. It's widely believed that formulations encapsulated in a polymeric matrix are more efficient than non-encapsulation ones. Among the polymeric controlled release types, nano and microparticles have shown a great potential for carrying and release an active ingredient (Figure 7) [9].

Figure 7 Polymeric nanoparticles for controlled release systems [9]

2.3.3 Chitosan

Several natural polymers are widely used in nanotechnology such as chitosan, alginates, polysaccharides cellulose, agarose, dextran and starch [5]. Among those, chitosan is one of the polymers commonly used in controlled release systems. It can be obtained by the extensive deacetylation of chitin, which is the second most abundant natural polymer on the planet, after cellulose [10], [37]. In order to do chitin deacetylation for chitosan synthesis, a hydrolysis process usually is done at alkaline conditions. Chitin can be found in the shell of crustaceans (shrimps, lobsters and crabs) and also in cells walls of fungi, cuticle of insects, radulae of molluscs and internal shells of cephalopods (octopus) [7], [38]**.** Chitosan, which was identified in 1859, can be found commercially with different degree of deacetylation and molecular weight [38]. Chemical structure of Chitosan compound it shown in Figure 8. This structure permit certain variations in the C-2 position for different applications, which represent a benefit in comparison to other polysaccharides [39].

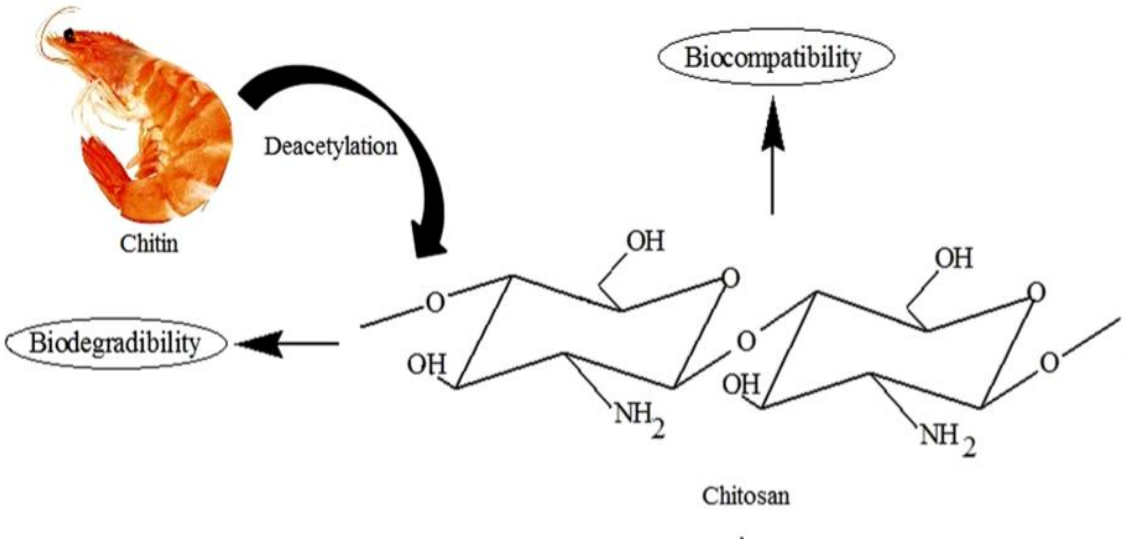

Figure 8 Chemical Structure of Chitosan [37]

Over the past few years, Chitosan has attracted the attention of the scientific community due its exceptional biological properties that can be used in several industries as medicine, pharmaceutics, food, cosmetics, water treatment, among others [10]. Lately, it has been demonstrated that Chitosan can also has a broad range of applications in the agriculture industry [40], [14]. Table 1, presents some of Chitosan applications in most of principal industries, including agriculture [39].

Chitosan is biocompatible, biodegradable, non-toxic and has low cost [11]**.** Besides, it have been reported that chitosan has antibacterial, antifungal and antivirus properties, which are very beneficial in agriculture [40]**.** Those unique properties, allows chitosan to be used as delivery matrix for controlled release of medication in humans and also of agrochemicals in agriculture activity [11]**.** Therefore, Chitosan can be suitable for the encapsulation of pesticides and as a controlled release system [11]**.** Table 1, summarizes chitosan applications in Agriculture and other areas [39].

Table 1 Principal Applications for Chitosan [39]

Agriculture	Defensive mechanism in plants
	Stimulation of plant growth
	Seed coating, Frost protection
	Time release of fertilizers and nutrients in soil
Water & Waste treatment	Flocculant to clarify water (drinking water, pools)
	Removal of metal ions
	Ecological polymer (eliminate synthetic polymers)
	Reduce odors
Food & beverages	Not digestible by human (dietary fiber)
	Bind lipids (reduce cholesterol)
	Preservative
	Thickener and stabilizer for sauces
	Protective, fungistatic, antibacterial coating for fruit
Cosmetics & Toiletries	Maintain skin moisture
	Treat acne
	Improve suppleness of hair
	Reduce static electricity in hair
	Tone skin
	Oral care (thoothpaste, chewing gum)
Biopharmaceutics	Immunologic, antitumoral
	Hemostatic and anticoagulant
	Healing, bacteriostatic

2.4 Literature Review: Controlled Release of Herbicides

In recent decades, there has been increasing attention in nanotechnology as a mechanism for enhance pesticides formulations without harming non-target organism. Various methods have been reported to produce nanomaterials as carrier's system for herbicides using chitosan and other polymers for agricultural applications.

Silva et al. 2011 [6], developed a system based of nanoparticles of chitosan and alginate loaded with the herbicide Paraquat. This non-selective contact herbicide, is commonly used in crops as coffee, sugar, cotton, apples among others, but is classified by EPA as very dangerous. In this case, microparticles were produced by a mixture of a chitosan solution and a second solution of alginate. After evaluation, it was found that Paraquat was incorporated successfully to the microarticle, showing 74% of efficiency. The particle of 635nm, release Paraquat 2 hours longer than free herbicide after 8 hours. A reduction in soil sorption profile was observed, thereby improving the activity of the herbicide. These finding suggest that chitosan/alginate particles have a potential use as a control release system allowing the decrease of environmental risks.

Grillo et al. 2012 [41], studied Polymeric poly(ε-caprolactone) (PCL) nanocapsules with three different herbicides (Ametryn, Atrazine, and Simazine) as a controlled release system. Nanocapsules were synthetized by an interfacial deposition of pre-formed polymer procedure. The efficiency of the encapsulated herbicides was more than 84%. The produced nanoparticles were spherical with uniform size in the range of 232nm-290 and formulations remained stables during 270 days. Herbicides associated with nanocapsules were less toxic than free herbicides, as well the release were delayed. This

controlled release systems could be use in order enhance the behavior of these herbicides for weed control.

Grillo et al., 2014, also synthetized chitosan nanoparticles as a carrier system for Paraquat herbicide by ionic gelification using sodium tripolyphosphate (TPP). The main goal of the research was generated an effective and non-toxic herbicide formulation for a securer weed management. Formulation toxicities evaluations were made in *Allium cepa* chromosome and Paraquat activity in maize and mustard. Chitosan/TPP nanoparticles with the herbicide reached a 62% of efficiency. Under laboratory conditions, release coming from 300nm particles were slower than the free herbicide. Results also shown a reduction in soil sorption and also that the herbicide linked with the nanoparticles was less toxic than pure compound, minimizing environmental impact.

Recently, Oliveira and colleagues [42] prepared nanocapsules of Poly(epsilon-caprolactone) (PCL) as a release system for the herbicide Atrazine. This pre- and post-emergence herbicide have been used as a technique to reduce weeds infestations in crops as sugarcane, maize and sorghum. Nevertheless, it is believed that the use of this formulation is related with water and soil contamination due its slow degradation. Besides, the excessive applications of this herbicide can cause adverse effects on some aquatic animals and plants and even in humans. The PCL nanocapsules were created with the objective of minimize the environmental contamination and enhance the herbicide action using mustard plants. In the herbicidal activity treatments, mustard plants leaves were sprayed with nanocapsules with Atrazine incorporated. Those plants showed signs of toxicity such as wilt and necrosis after seven days. Instead to plants treated with nanocapsules without Atrazine which no shown any toxicity. Results indicated that

nanoencapsulation increase the biological activity of the herbicide, suggesting that Atrazine dosages can be reduced without affecting the good performance of the herbicide. Also, it was found that the encapsulation of the herbicide decreases its mobility in the soil, therefore minimize water contamination.

In 2016, Maruyama *et al.*, [43] published another study in which synthetized chitosan microparticles with alginate or tripolyphosphate as carriers for the combined herbicides of Imazapic and Imazapir. These herbicides are applied in peanut, soybean and corn to control a broad spectrum of weeds. Lately, it has begun to apply these herbicides together as an alternative to resistance issues. Therefore, this research arises with the intention of improve the application techniques of the herbicides of Imazapic and Imazapir. Both microparticles approximately of 400nm, presented a good encapsulation efficiency over the 60%. Importantly, release assays indicated a slower release of the combine herbicides encapsulated in comparison to the free agents with the ALG microparticles. The herbicides associated to the microparticles resulted be more effective and shown low toxicity. These findings reveal the chitosan microparticles as an effective and environmental friendly release system for herbicides.

From the above considerations, it can be seen a significant progress in controlled release systems for herbicides in the last few years. Despite that, only a minimum number of herbicides have been study for this purpose [36]; there are no attempts to study the potential of chitosan nanoparticles as controlled release of the herbicide Bromacil. In addition, the method proposed by the literature for the nanoparticle synthesis was used for a fertilizer, not for an herbicide, which involves a different chemical structure. Accordingly, it is of key importance for the treatment of agriculture soils to assess the

capability of using biocompatible polymeric nanobeads loaded with the herbicide and, hence, improve weed control under environmental protection conditions.

3. Objectives

3.1. Main

- Determine optimum synthesis conditions of chitosan based nanoparticles with different sizes

3.2. Specific

- Characterize the nanoparticle on chemical structure, morphological and functional bases
- Determine the optimum conditions for the incorporation of the herbicide to the nanoparticle
- Determine the conditions for the release of the herbicide compound

4. Experimental

4.1. Materials

All reagents were of analytical grade and used without any further purification. Require concentrations of Chitosan powder (≥75% deacetylation, Sigma Aldrich) and methacrylic acid ($C_4H_6O_2$, 99%, Alfa Easer) were added to high purity water. Methacrylic acid was used to dissolve the polymer chitosan. Potassium persulfate ($K_2S_2O_8$, 99% Alfa Easer) was used as the polymerization initiator and Bromacil ($C_9H_{13}BrN_2O_2$, Sigma-Aldrich) as the herbicide. Sodium Hydroxide (NaOH, 98%, Alfa Aesar) was dissolved in high purity water and used to precipitate the chitosan particles.

4.2. Size-Controlled Synthesis of Chitosan Nanoparticles (CS-PMAA)

Chitosan nanoparticles (CS-PMAA) were synthesized based on Moura *et al [44]* procedure, via the methacrylic acid (MAA) polymerization of a starting chitosan solution. Chitosan powder was added to a methacrylic acid aqueous solution 0.5% (v/v) under continuous stirring for 24 hours. Three concentrations of chitosan (0.2, 0.5, 0.8 wt.%) were synthetized for size controlled and to determine the optimal concentration. Then, 0.2 mmol of $K_2S_2O_8$ was added to the CS-MAA solution and stirred for 5 hours. After the mentioned period, the solution was stirred and heated at 70 ^oC for 1h. Finally, the nanoparticles' solution was quenched in an ice bath at 4 ^oC. The nanoparticles were precipitated using a NaOH solution and centrifugation at 8,000 rpm for 30 min. The precipitate was washed three times with deionized water and dried at 25^oC for 24 hours for characterization.

4.3. Characterization of Chitosan Nanoparticles (CS-PMAA)

4.3.1. X-Ray Diffraction (XRD)

X-Ray diffraction is an analytical technique used to study the molecular structure of a material. The spectra produced by the diffraction of X-Rays offer information about crystallinity and grain size, sample purity, among others. This technique is based on generation of the X-Rays in a cathode ray tube, which are filtered to produced monochromatic radiation. These X-Rays are directed toward the sample and then the diffracted rays are collected. The emitted X-Rays interact with the sample generating a constructive interference (Figure 9), which can be described by Bragg's Law [45]:

$$n\lambda = 2d \sin\theta$$

Where n = the order of reflection

λ = wavelength of incident ray

d = interplanar distance of the crystal

θ = angle of incidence and reflection of incident ray (Bragg's angle)

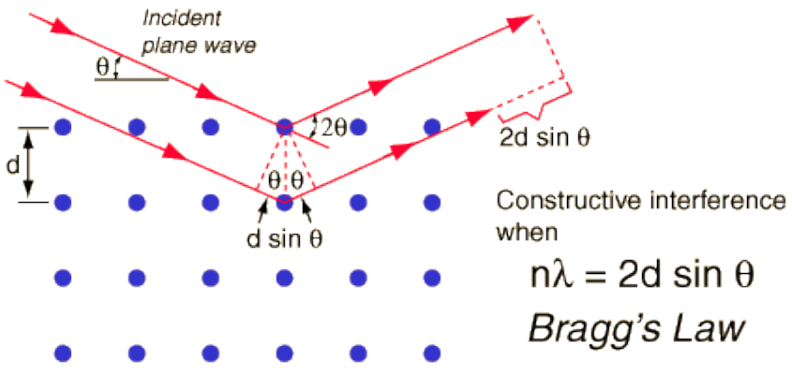

Figure 9 Schematic of diffraction and Bragg's Law [46]

A Siemens D5000 X-Ray Diffractometer (XRD) with Cu K_α radiation was used in order to identify the crystalline structure of CS-PMAA nanoparticles. The XRD is located in UPR Mayaguez at the Engineering Science and Materials Department (Figure 10). All spectra were taken from 15° to 75° degree and a step of 0.02.

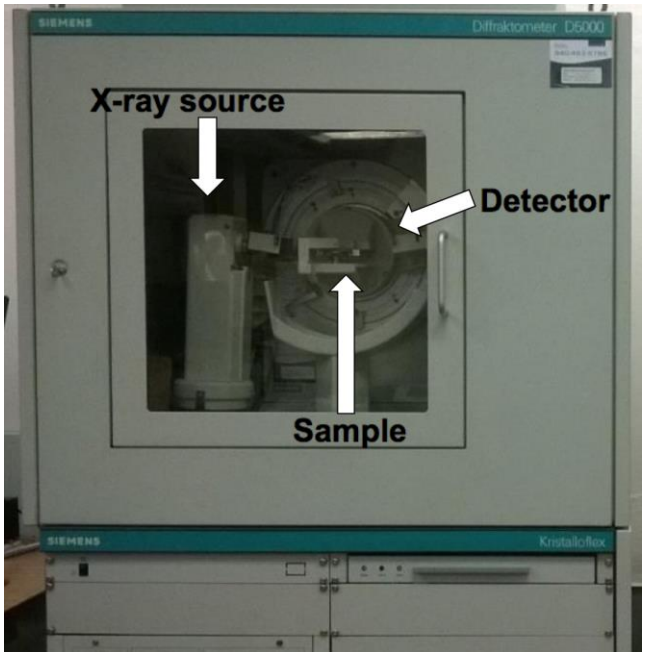

Figure 10 Siemens D500 powder X-Ray Diffractometer

4.3.2. Fourier Transformed Infrared Spectroscopy (FTIR)

Infrared Spectroscopy is a method used for materials analysis used to identify molecular bonds and functional groups of the sample (solid, liquid or gas) using infrared radiation. The infrared spectrum surge when a sample is exposed to infrared radiation and the sample molecule absorbs a particular energy, which correspond to the frequency of a vibration of the chemical bonds. Analyzing the infrared spectrums its possible identify a sample [46], [47].

The FTIR analysis were taken in a Shimadzu IRAffinity-1 Infrared Spectrophotometer located at the Engineering Science and Materials Department at UPRM (Figure11). Attenuated total reflectance (ATR) mode were used to measure the samples. In this mode, the beam from the source reflects the sample and passes to the detector, thus the spectra are taken from the surface of the sample. All spectra were taken in the spectral range of 4000 - 400 cm^{-1} by accumulation of 200 scans at resolution of 4 cm^{-1}.

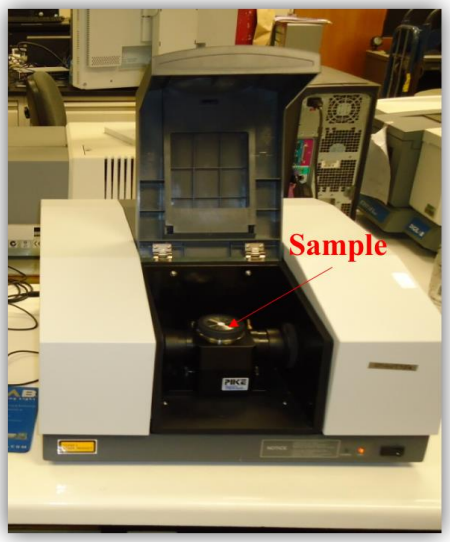

Figure 11 Shimadzu IRAffinity-1 Fourier Transformed Infrared Spectrophotometer (FT-IR) at UPRM

4.3.3. Zetasizer Nano

The Zetasizer Nano is an instrument used for measurement of the size and zeta potential of nanoparticles and colloids. It's also used for study some characteristics of proteins as their electrophoretic mobility and the molecular weight of macromolecules among other things [48]. For size measurements, the equipment uses the Dynamic Light Scattering (DLS). This technique measures the diffusion particles moving under Brownian motion and transform this to size. The intensity fluctuations in the scatter light can be studied by the illumination of the particles with a laser.

For Zeta potential measurements is used a Laser Doppler Velocimetry for determinate the Electrophoretic Mobility [49]**.** When a dispersion of particles is exposed to an electric field, they moved with a velocity related to their Zeta potential. This velocity allows to calculate the Electrophoretic Mobility and then the zeta potential. The Zeta Potential is the electrical potential that exists in the interfacial double layer of a dispersed particle. A MPT-2 Autotritator accessory is used to study the effect of changes in pH and assist with zeta potential measurements.

The particle size and zeta potential measurements of the Chitosan nanoparticles were performed in a Zetasizer NanoZS (Malvern Instruments) located at the Engineering Science and Materials Department at UPRM (Figure 12).

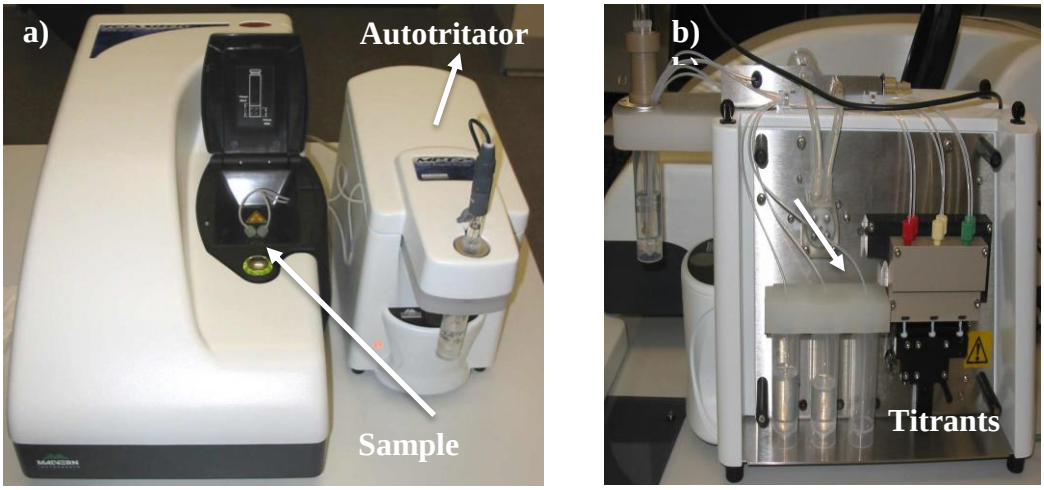

Figure 12 (a) Zetasizer NanoS with the MPT-2 Autotritator (b) MPT-2 Autotritator Accessory with titrant containers for acids or bases.

4.3.4. Transmission Electron Microscope (TEM)

The Transmission Electron Microscope (TEM) is an analytical technique that allows the study of samples in the field of micro space to nanoscale. Hence, this type of microscope has become a key component to characterize nanoscale materials and devices. Some of the materials that can be study in the TEM are metals, ceramic, polymers, semiconductors, among others. TEM uses an electron beam to produce images of a sample with high-resolution and magnification [50],[51]

A JEM-ARM200cF Transmission Electron Microscope was used to characterize Chitosan nanoparticles. Images were performed by the TEM of the Florida State University Research Foundation (Figure 13).

Figure 13 JEM-ARM200cf Transmission Electron Microscope on Florida State University [53].

4.4. Synthesis of Chitosan Nanoparticles loaded with Bromacil

The synthesis of chitosan nanoparticles loaded with Bromacil was achieved by using two methods in order to find the best way of incorporating the nanoparticle with the herbicide. The chitosan concentration used for these syntheses were 0.2 wt.%. In the first method (A) Bromacil solution were added in the beginning of the nanoparticle synthesis after the chitosan was added to the methacrylic acid solution. The subsequent procedure was as described in part 3.2. In the second method (B), after the preparation of the nanoparticles Bromacil solution was added to nanoparticle precipitate in an aqueous solution stirring for 24 hours. Four concentrations of Bromacil (60, 100, 200, 480ppm) were added to the particles. Figure 14 summarizes both synthesis routes.

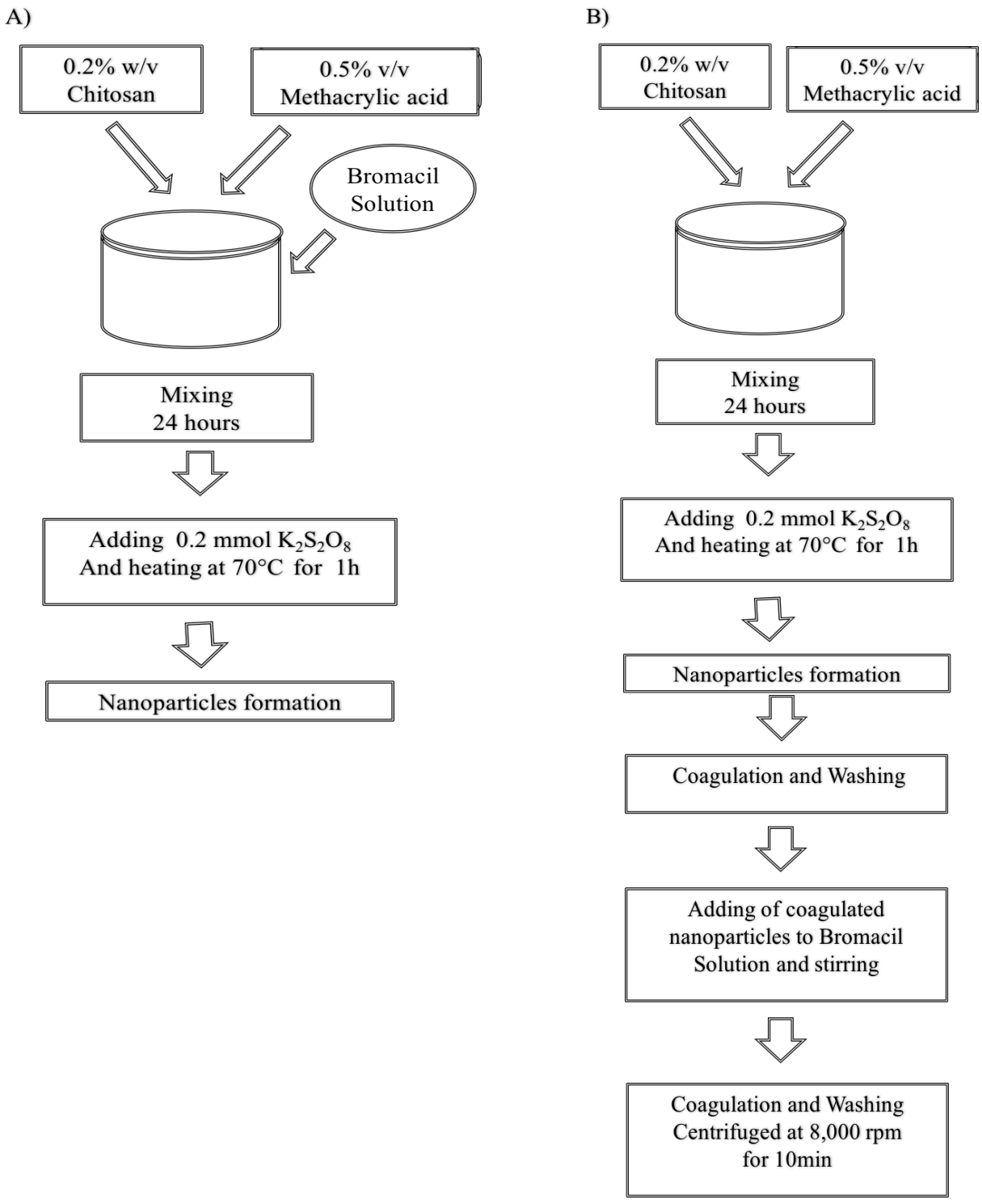

Figure 14 Synthesis method of Chitosan Nanoparticles adding Bromacil at the beginning (A) and at the end (B) of the process.

4.4.1. Efficiency of encapsulation of Bromacil in Chitosan nanoparticles

The amount of herbicide in the nanoparticle was determined by a mass balance between the initial Bromacil concentration in starting solutions and the filtrate generated after the Chitosan nanoparticles were synthesized. In method A, once formed Chitosan nanoparticles in suspension were coagulated with a NaOH 0.5M solution and centrifuged for 30 minutes to recover the solid fraction containing the nanoparticles (Figure 15). The suspension supernatant was passed through a 0.45 μm nylon membranes and the solution was analyzed using a High-Performance Liquid Chromatograph (HPLC). In method B, the supernatant analyzed in HPLC was the resulted from the second coagulation process. In order to quantified the herbicide, a calibration curve was prepared describing the association between Bromacil concentrations and the HPLC detector responses.

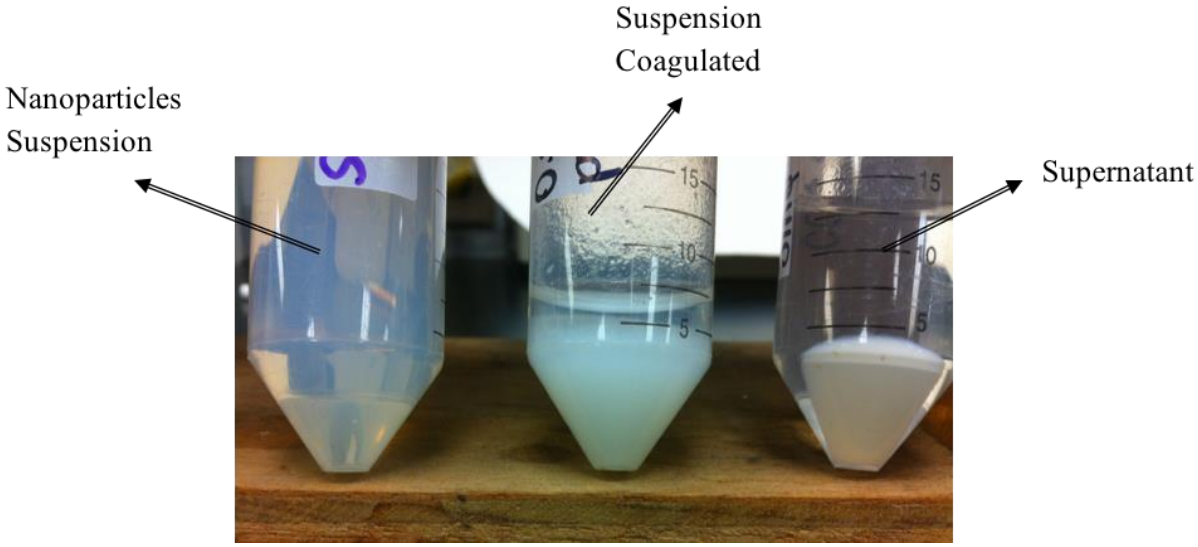

Figure 15 Nanoparticles suspension before and after coagulation process in Method A

4.4.2. High-Performance

The High-Performance Liquid Chromatograph (HPLC) is an analytical technique used to separate the components of a mixture, to identify each component and quantify them. This technique involves an injection of a small volume of liquid sample into a column packed with porous particles (stationary phase). The components of the mixture are transported along the column by a liquid (mobile phase) and are separated from one another by chemical or physical interactions between their molecules and the packing particles in the column. After the separation, the resulted chromatogram offers qualitative and quantitative information about each compound. This type of chromatography is sensitive and accurate and non-destructive [52],[53].

A High-Performance Liquid Chromatograph Diode Array Detection (HPLC-DAD) was used to analyze Bromacil in Chitosan nanoparticles (Figure 16). This HPLC it's equipped with a quaternary pump system. A column Agilent Eclipse EDB C-8 (Dp, 5um: 150x25cm) were used. The co-reverse mobile phase consisted of Acetonitrile-Water (70:30 v/v), both solvents HPLC grade performance at a flow rate of 1.0 mL/min. The detection was carried out at 271 nm.

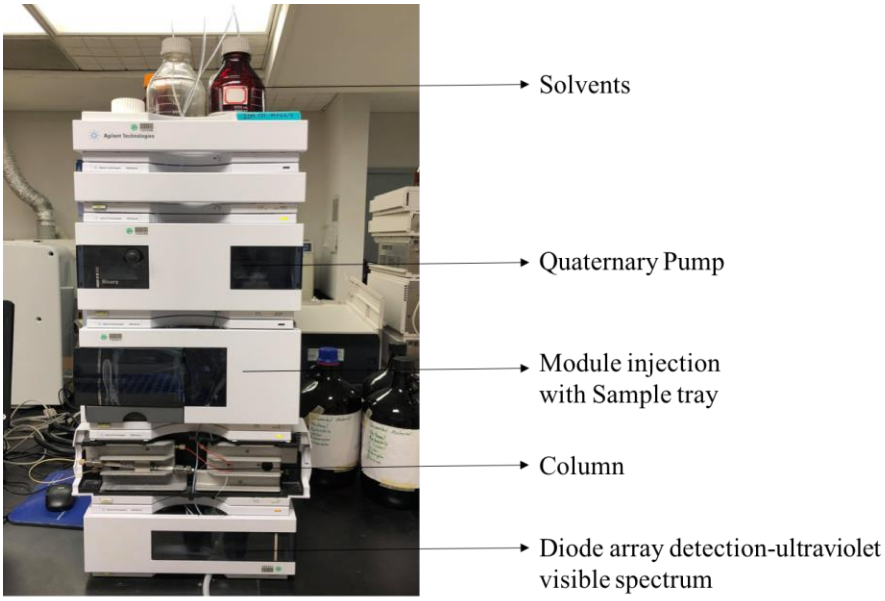

Figure 16 HPLC located at the Department of Chemistry at UPRM

4.4.3. In vitro assays of Bromacil Release from Loaded Chitosan into the aqueous phase

For release assays, the nanoparticles synthesis method used was the Method A due better incorporation of Bromacil. The release of the Bromacil from nanoparticles were quantified using a dialysis-like system of two compartments (donor and acceptor) with a Float-A-Lyzer G2 Dialysis device from Spectrum Labs (Figure 17), under agitation. This device has a cellulose membrane with a molecular exclusion pore size of 0.5-1.0 kDa. The atomic mass unit Kilodalton (kDa) is used to define the molecular weight of macromolecules. The donor compartment had the nanoparticle loaded with Bromacil and the acceptor compartment an aqueous solution. Aliquots from the acceptor compartment was periodically taken evaluated by HPLC. The peak area of the HPLC was transformed into the percentage of Bromacil released.

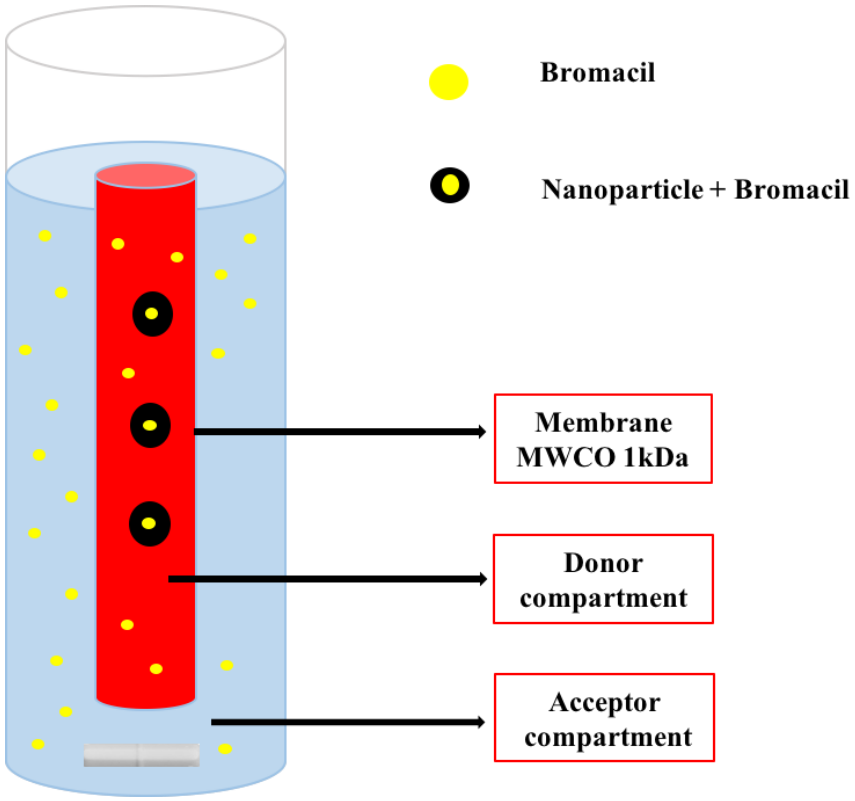

Figure 17 Schematic representation of the Release Experiment with the Nanoparticles suspension in the donor compartment [56]

4.4.4. Mathematical Model

The Korsmeyer–Peppas model [54] was applied to the release curves of Bromacil release from the chitosan nanoparticles in order to identify the type of mechanism involved. The model is described by:

$$M_t / M_\infty = Kt^n$$

where, M_t is the amount of herbicide release at time t

M_∞ is the total amount of herbicide releases at time t

K is the kinetic constant,

n is the exponent (which reflects the type of release mechanism)

Korsmeyer and Peppas proposed that values of $n \leq 0.43$ are indicative of release mechanisms that follow diffusion Fick's Law, while $n > 0.85$ indicates that the mechanisms are governed by relaxation processes, defined as Case II type transport. Intermediate values ($0.43 < n < 0.85$) suggest anomalous behavior, with non-Fickian release kinetics (and a combination of diffusion and relaxation of the polymeric chains).

5. Results and Discussion

5.1. Size-Controlled Chitosan nanoparticles (CS-PMAA)

5.1.1. Structural Characterization

5.1.1.1. X-ray Diffraction Analyses

Figure 18 shows the XRD patterns of chitosan powder and CS-PMAA nanoparticles synthesized using 0.2 wt.% chitosan concentration. As seen, broad diffraction peaks were observed at 12.5° and 20° corresponding to (020) and (110) crystallographic planes, respectively, suggesting the semi-crystalline nature of the polymer. In turn, the pattern corresponding to the CS-PMAA nanoparticles exhibits a unique and very broad peak centered on 13°. The observed difference between the XRD patterns of chitosan powder and nanoparticles may be attributed to the movement of the polymer chains during the synthesis process and decrease in crystallinity at the nanosize level.

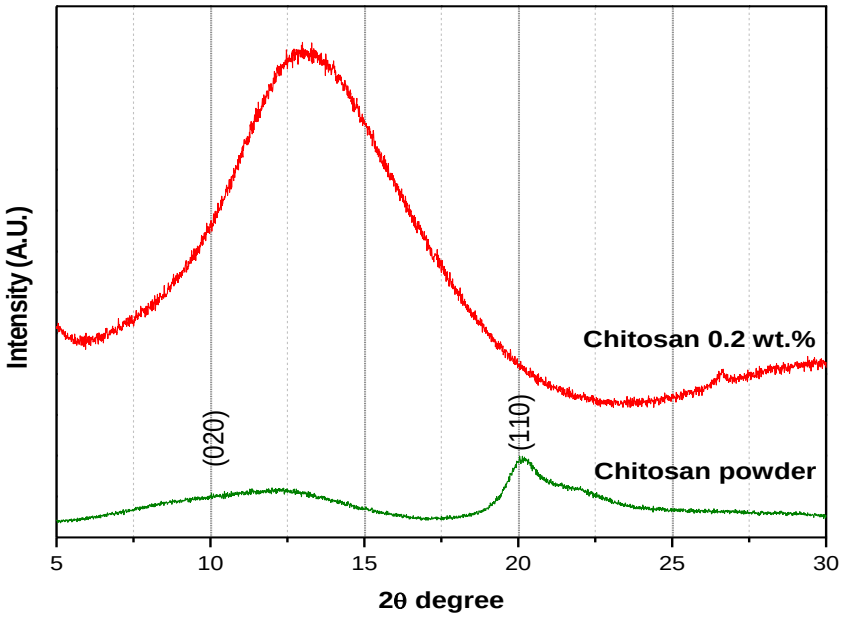

Figure 18 XRD patterns corresponding to chitosan powder precursor and CS-PMAA nanoparticles synthesized form starting 0.2 wt.% solutions

5.1.1.2. Fourier Transform Infrared Spectroscopy (FTIR) Measurements

The FT-IR spectra for chitosan powder and CS-PMAA nanoparticles synthesized at different CS concentrations (0.2, 0.5, and 0.8 wt.%) are shown in Figure 19. The spectrum of the CS powder precursor (Figure 2a) showed characteristics bands at 1650 cm^{-1} , attributed to a C=O stretching vibration of amide I [54], 1070 cm^{-1} related to C-O-C stretching of skeleton, and 1150 cm^{-1} associated to antisymmetric C-O-C stretching of glycosidic link [37]. CS-PMAA nanoparticles spectra (Fig. 2b, 2c and 2d), show two new bands at 1545 and 1638 cm^{-1} attributed to NH^{+}_{3} and COO^{-} groups, respectively. These two bands are related to the nanoparticles formation, suggesting an ionic interaction between CS and PMAA [44]. The presence of bands at 1264 and 1703 cm^{-1}, corresponding to C=O, indicate the existence of PMAA in the chitosan nanoparticle. Both bands (1264 and 1703 cm^{-1}) decrease in intensity with increasing chitosan concentration, due to reduction of PMAA in the nanoparticle. This analysis confirmed the formation of the chitosan nanoparticles, which can be used for use for controlled-release applications.

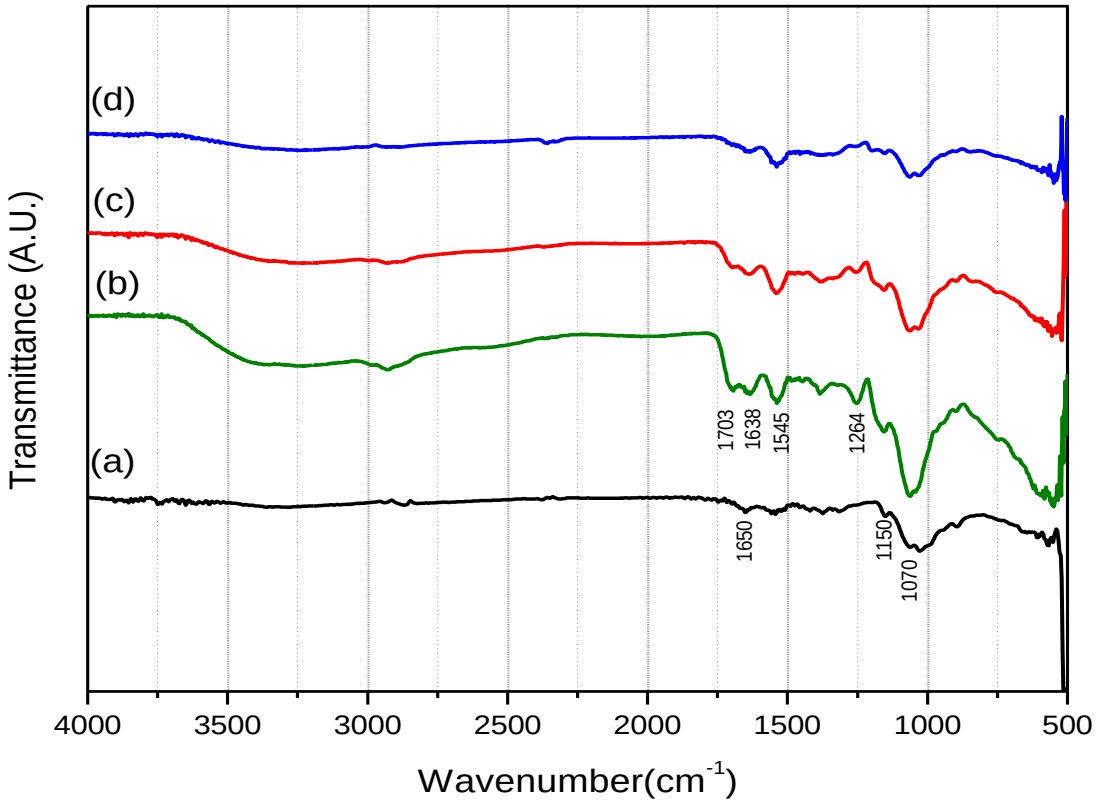

Figure 19 FTIR spectra of: (a) CS powder and CS-PMAA nanoparticles synthesized with (b) 0.2, (c) 0.5, and (d) 0.8 wt.% of CS

5.1.2. Physicochemical Characterization

5.1.2.1. Hydrodynamic diameter and Zeta Potential Analyses

Table 2 shows the hydrodynamic diameter values of CS-PMAA nanoparticles synthesized at different CS concentrations and suspended in their mother liquors. As observed, Chitosan nanoparticles with different hydrodynamic diameters could be obtained varying the chitosan concentration. Nanoparticles prepared with 0.2 wt% CS, exhibited the smallest diameter (59 nm). A general trend shows that the hydrodynamic diameter increases as the CS concentration increases. These findings demonstrated a tuning in particle size, which is highly important at the nanoscale [55].

Table 2 Hydrodynamic diameter values of CS-PMAA nanoparticles synthesized at 0.2, 0.5, and 0.8 wt %

CS concentration (wt%)	Hydrodynamic diameter (nm)
0.2	59±0.5
0.5	103±1
0.8	149±2

The zeta potential of suspended CS-PMMA nanoparticles were measured as a function of the suspension pH (Figure 20). The variation in the zeta potential measurements with pH are closely related to the changes in the electrical charges (positive or negative) on the surface of the particle [12] . The positive values of zeta potential, which vary for each CS concentration, indicate that nanoparticle surface are positively charged because of the chitosan cationic characteristics, as reported by Moura *et al* [44]. The increase in zeta potential values at larger CS concentration can be attributed to the fact that there are more groups NH_3^+ from chitosan in comparison with

the COO^- groups of the methacrilyc acid. The isoelectric point for the 0.2, 0.5 and 0.8 wt.% CS nanoparticles are 5.46, 5.54, and 7.29, respectively. At this point, the positive and negative surface charges are equal and the system becomes unstable and coagulates. Usually, when the zeta potential exceeds the ±30 mv means that the system is in a disperse state [6]. The observed negative zeta potential value under less acidic and alkaline conditions is the result of the ionization of the COO^- groups of the methacrilyc acid and the neutralization of the NH_2 groups of chitosan [12] .

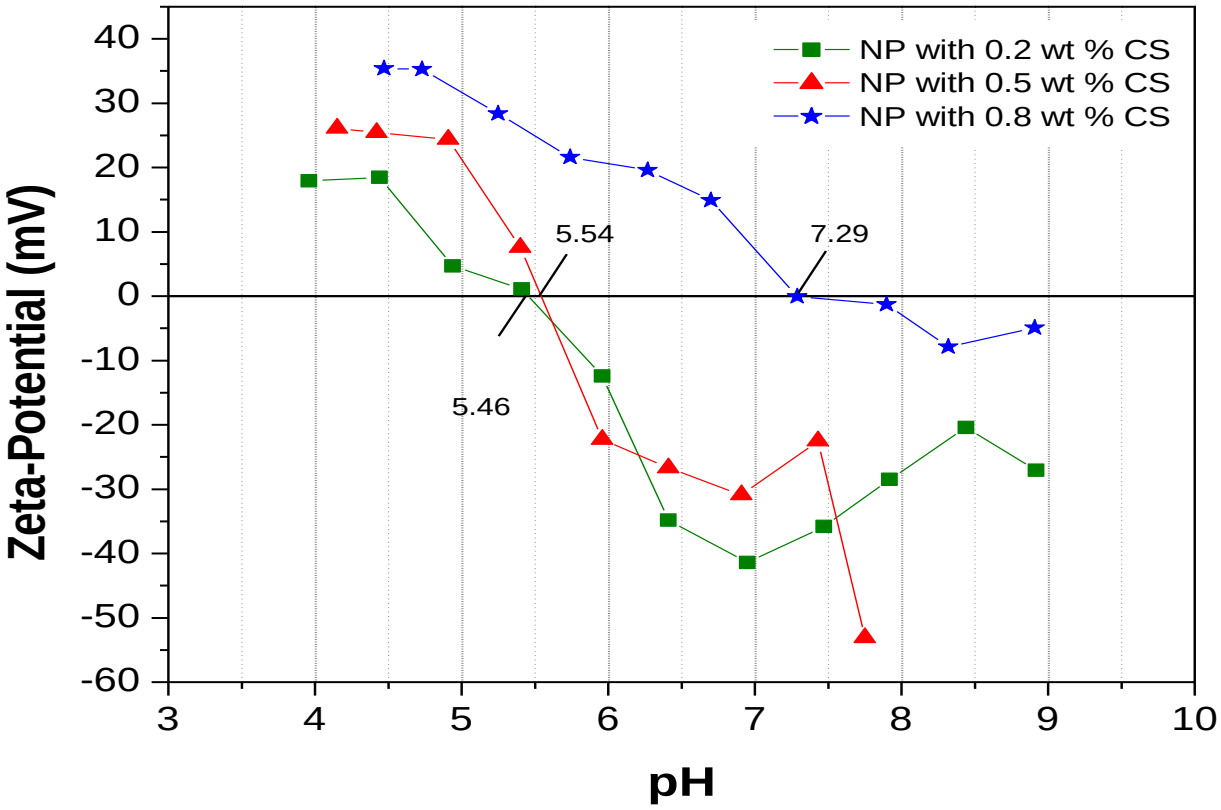

Figure 20 Zeta potential as a function of the pH for CS-PMAA nanoparticles prepared with 0.2, 0.5, and 0.8 wt.% CS

5.1.3. Morphological Characterization

5.1.3.1. Transmission Electron Microscopy Analyses

TEM images of CS-PMAA nanoparticles synthesized at 0.2 wt.% CS are presented in Figure 21. The nanometric size of the rather spherical shaped CS-PMAA nanoparticles was confirmed (Figures 21 a-c). Figure 22 shows the CS-PMAA nanoparticles size distribution histogram; it was found that the greater number of nanoparticles is in the range of 6.0 and 8.0 nm for an average of 17.7 ± 0.7 nm.

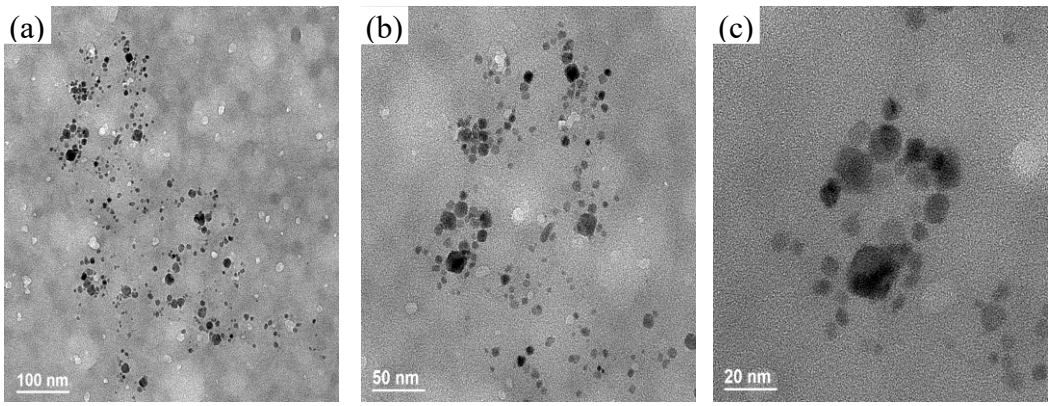

Figure 21 (a-c) TEM images of CS-PMAA nanoparticles synthesized at 0.2 wt.%.

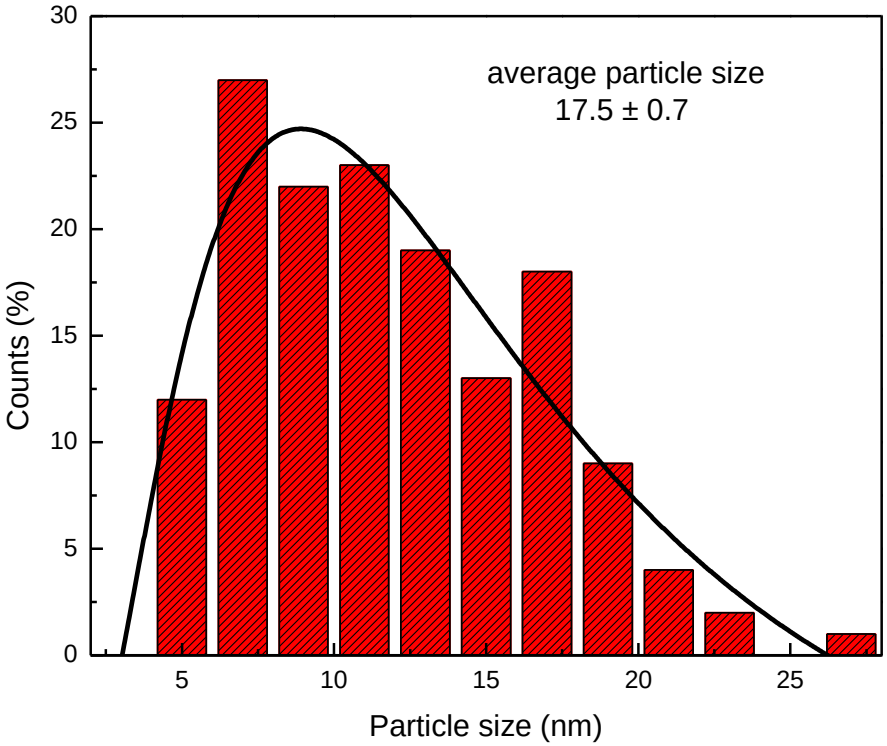

Figure 22 CS-PMAA nanoparticles (0.2 wt.%) size distribution histogram

5.1.4. Remarks

Chitosan nanoparticles were successfully synthesized by the polymerization of methacrylic acid. Nanoparticles with different sizes were successfully synthesized varying CS concentration. X-Ray Diffraction and FTIR confirmed the formation of the nanoparticles and the interaction between the COO^- groups of methacrylic acid and the amino groups of chitosan. Zeta potential analyses indicate the pH-dependence of the CS-PMAA nanoparticles surface charge; the observed negative zeta potential value under less acidic and alkaline conditions is attributed to the ionization of the COO^- groups of the methacrylic acid and the neutralization of the NH_2 groups of chitosan.

5.2. Synthesis of Chitosan Nanoparticles loaded with Bromacil

5.2.1. Encapsulation efficiency

Nanoparticles have a high surface area to volume ratio, which allows the association with other substances. However, this association depends of several factors as the nanoparticle preparation method, the chemical nature of the substance and its polarity [56]. An active substance can be added to nanoparticles at the time of preparation of the nanoparticle or after the particles formation (Method A and B).

The encapsulation efficiency (EE%) is defined “by the concentration of the incorporated material (such as active ingredients, drugs, pesticides) detected in the formulation over the initial concentration used to make the formulation” [57]. The encapsulation efficiency obtained for the chitosan nanoparticles containing different concentrations of the herbicide Bromacil for both methods used (A and B) are compared in Table 3. The values obtained for method A were 62.56% (60ppm), 62.32%, (100ppm), 57.42% (200ppm) and 52.88% (480ppm). All four concentration association values for Method A, were greater than the obtained by Method B, where the higher value was 54.12% for the 100ppm herbicide concentration. These findings agree with literature, that report that incorporation efficiency can be increased when the active ingredient is added during the formation of the nanoparticles [58]

Table 3 Encapsulation efficiencies (EE%) of nanoparticles with different quantities of the herbicide Bromacil added at the beginning (Method A) and at the end (Method B) of the synthesis

Bromacil Concentration (ppm)	Method A (%)	Method B (%)
60	62.56	52.73
100	62.32	54.12
200	57.42	47.79
480	52.88	45.70

Previous works ([4], [41], [43]) of nanostructured systems with different herbicides as Paraquat, Atrazine and Imazapyr, obtained EE% around 50 to 60% and others obtained values above the 80%. The variability of these results could be explained in part by the difference between the herbicides water solubility. It's known that herbicides with low water solubility (hydrophobic) can have better affinities with the nanoparticles than those with a high water solubility (hydrophilic) [56]. On the other hand, it's important to mention that for herbicides controlled release systems it can be favorable that certain amount of the herbicide stay free in order to eliminate weeds in the beginning of the process [4].

Chitosan nanoparticles showed good encapsulation efficiency for both methods used indicating its potential as controlled release systems. Nevertheless, due the better incorporation of Bromacil with nanoparticles by method A was selected for the release experiments.

5.2.2. Characterization of Nanoparticles

5.2.2.1. Structural Characterization

5.2.2.1.1. Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectra for Bromacil powder and chitosan nanoparticles synthesized with different concentrations of Bromacil (60, 100, 200 and 480ppm) are shown in Figure 23. The spectrum of the Bromacil molecule showed characteristics bands at 1710 cm^{-1} attributed to a C=O, 3070 cm^{-1} and 840 cm^{-1} possibly associated to C-H stretching vibrations bond. The chitosan nanoparticles spectrum show bands at 1543 and 1703 cm^{-1}, corresponding to the NH^{+}_{3} and C=O respectively, related to the interaction between the chitosan and Poly(methacrylic acid). In the case of the different spectrum of the nanoparticles with Bromacil, the band at 1543 NH^{+}_{3} related to the chitosan is present but the characteristics herbicide absorption bands do not appear. This could be due to be indicative of a possible interaction between the Bromacil groups and the chitosan chains, changing the herbicide functional group vibrational frequencies, which become superimposed onto those of the nanoparticle polymer [59].

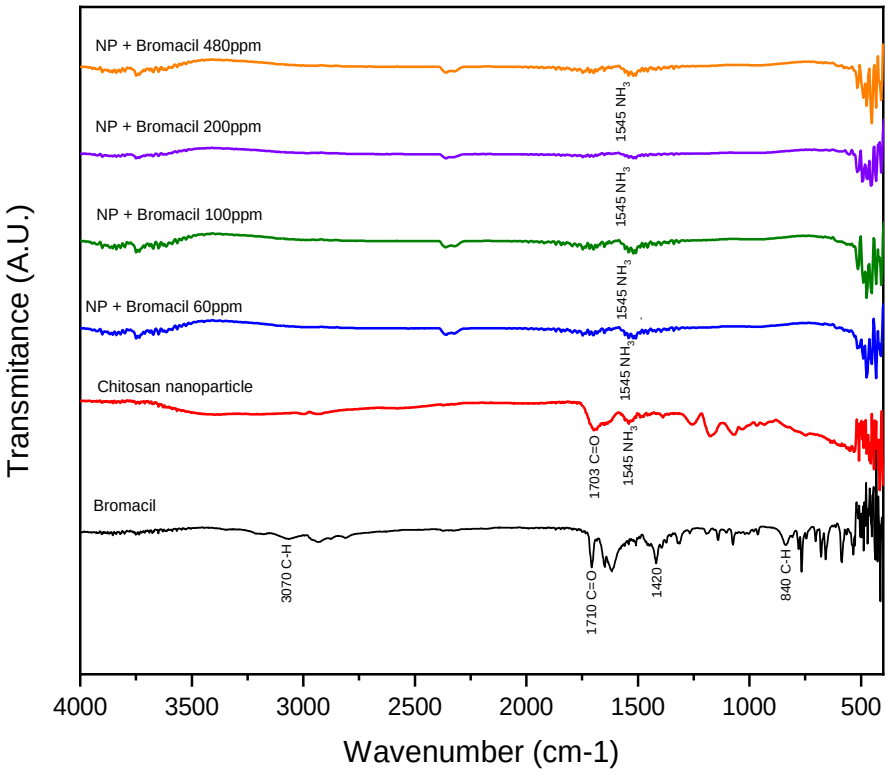

Figure 23 FTIR spectra of Bromacil powder, chitosan nanoparticle and nanoparticle with Bromacil with 60, 100, 200 and 480ppm

5.2.2.2. Physicochemical Characterization

5.2.2.2.1. Hydrodynamic diameter and Zeta Potential

Table 4 shows the hydrodynamic diameter values of chitosan nanoparticles synthesized with different Bromacil concentrations (60, 100, 200, 480ppm) suspended in water. As observed, all chitosan nanoparticles with Bromacil presents hydrodynamic diameters around 60nm. These values are similar to those of empty nanoparticles (~59nm) prepared with 0.2 wt% CS, showing that incorporation of the herbicide did not affect the particle size [4], [41].

Table 4 Hydrodynamic diameter values of chitosan nanoparticles (0.2wt%) synthesized with different Bromacil concentrations

Bromacil concentration (ppm)	Hydrodynamic diameter (nm)
60	62±0.3
100	60±0.6
200	65±1.5
480	63±0.1

The stabilities of the nanoparticles suspensions with Bromacil were evaluated using measurements of Zeta potential. Figure 24 shows the zeta potential of suspended chitosan nanoparticles with different concentrations of Bromacil as a function of the suspension pH (Figure 25). The values of the zeta potential at initial suspension pH (3.5-4) were positive due the cationic characteristics of the chitosan and were around 18mV. These values are similar to the ones obtained of nanoparticles without the herbicide.

However, zeta potential values of the samples with 200 and 480ppm of Bromacil resulted in increased stability, with higher zeta potential (+26mV and 24mV) respectively. Nanoparticles with zeta potential values near ±30 mv are mostly more stable in suspension [44]. Maruyuma et al., 2015 [43], reported that the incorporation of certain herbicides to a chitosan nanoparticle can improve the stability of the suspension, preventing particle aggregation with time.

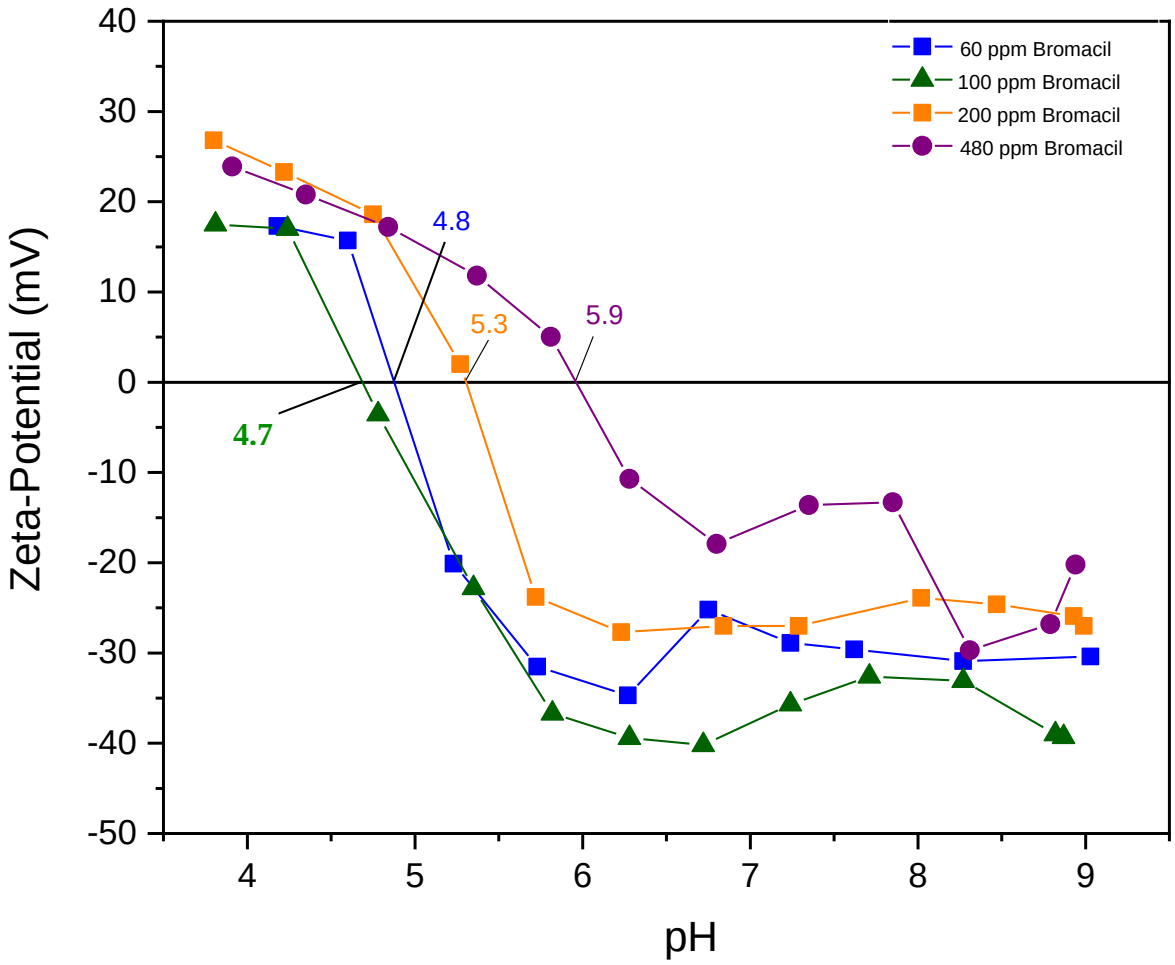

Figure 24 Zeta potential as a function of the pH for chitosan nanoparticles prepared with 60, 100, 200 and 480ppm of Bromacil

5.2.2.3. Herbicide Release

The release of a substance from a nanoparticle depends of many factors, such as the concentration and physicochemical characteristics (e.g. solubility) of the substance. It's also depend of the nanoparticle size; nature, molecular weight and concentration of the polymer. Besides, parameters as the medium pH and temperature, contact time and preparation method can greatly influence the release rate of the substance [56].

Assays of Bromacil release profile were performed to evaluate the release of the herbicide with the nanoparticle in comparison of the release of the free herbicide (without nanoparticle), as a function of time. The HPLC allowed detect the Bromacil concentrations after the assays was finished. A calibration curve was made with diluted Bromacil solutions ranging from 0.5 to 40ppm. Figure 25 shows a direct relationship between the standard Bromacil concentration and the area detected by the HPLC represented in the chromatogram. The linear regression model obtained, with a correlation coefficient R^2=0.9998, was the following:

$$\mathbf{y = 74.331x - 4.7319}$$

Where: x = concentration of Bromacil in solution (ppm)

y= area under de curve represented in the chromatogram (AU)

The limit of detection and limit of quantitation calculated were 0.27ppm and 0.83ppm respectively.

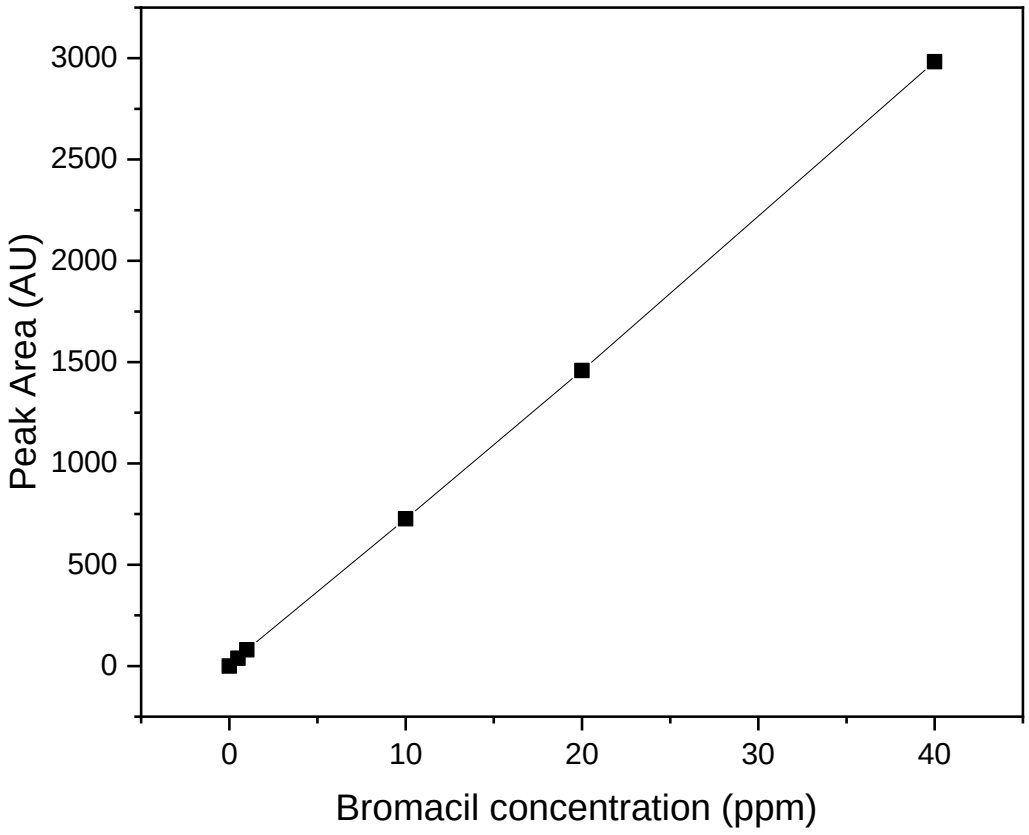

Figure 25 Calibration curve for Bromacil concentrations using the HPLC technique

The unknown concentration of Bromacil was determined using this expression. The results from the HPLC were plotted as the percentage of herbicide release from the nanoparticles as a function of time. This measure could be evaluated because only Bromacil molecules were able to pass through the pores of the membrane.

5.2.2.3.1. Effect of Bromacil Concentration

The percentage release curves for Bromacil concentration of 80 and 100ppm, either free or associated with the nanoparticle are presented in Figure 26 and Figure 27, respectively. For the 80ppm concentration, 3.7% of the free herbicide was available in the acceptor compartment after 120 hours, while during the same time period 3.3% of the herbicide related to the nanoparticle was released. In the other hand, the experiment with concentration of 100ppm, shown 2.7% release of the free herbicide after the 120 hours, while the Bromacil associated with the nanoparticle had 2.6% during the same period. There was no significant difference between the two concentrations (80 and 100ppm), which can probably be explained by the similarity of both concentrations. It seems, the initial concentration of the herbicide had no effect on the EE% or release. However, the study shown that there is a tendency that the herbicide associated to the nanoparticle resulted in slower release, compare to the free compounds.

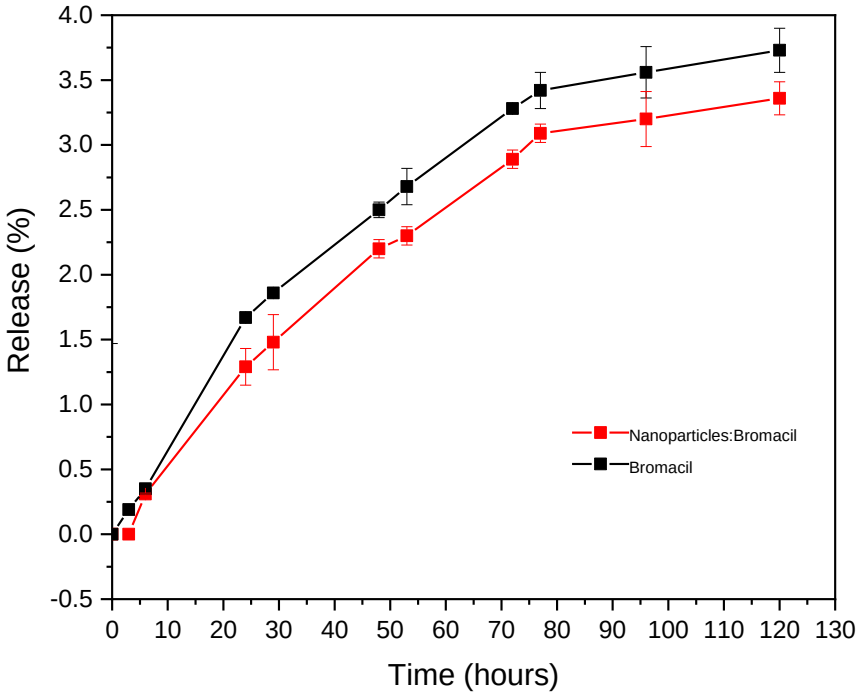

Figure 26 Release profiles in water of free Bromacil and Bromacil associated with the chitosan nanoparticles at 80ppm at 25°C

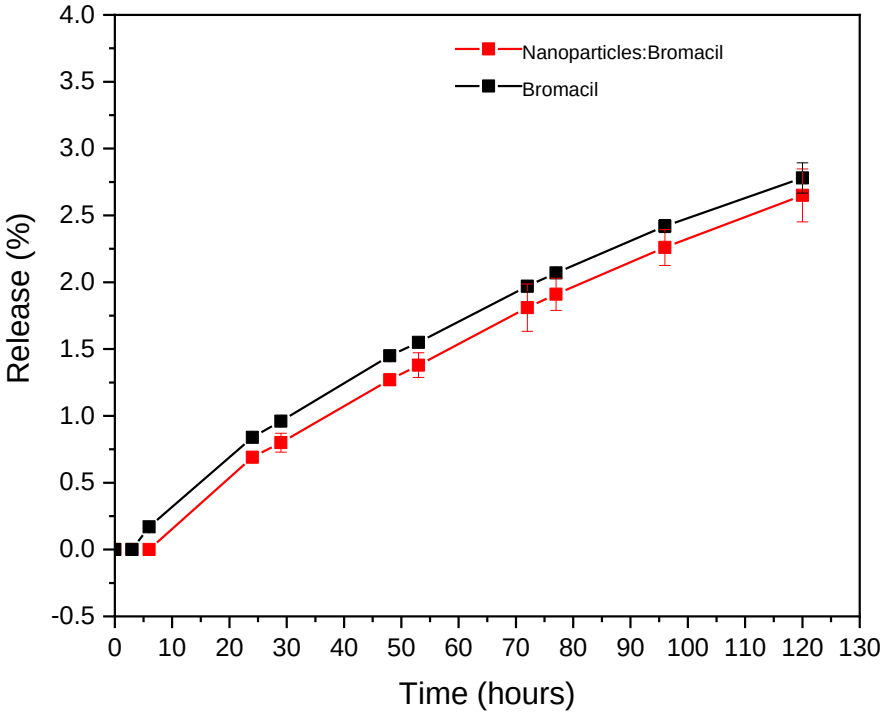

Figure 27 Release profiles in water of free Bromacil and Bromacil associated with the chitosan nanoparticles at 100ppm at 25°C

5.2.2.3.2. Effect of pH and Temperature

The pH and the temperature were evaluated to assess how the release of Bromacil (100ppm) from nanoparticle can be effected by those two parameters. The release percentage of Bromacil coming from the nanoparticle with pH (9.5-10) in the receptor compartment is presented in Figure 28. The maximum release rate was 3.3% at 120 hours, 0.7% more than the release from nanoparticle without pH changes at the same time period. This result suggests that the release of Bromacil from the nanoparticle, can't be controlled by pH changes.

In contrast, the assays of nanoparticles with Bromacil exposed to 40°C of temperature revealed 6.6% of release at 120 hours (Figure 29). This represents an increase of 3% of the release compared with the release with the nanoparticles exposed to room temperature. The increase in the release percentage can be attributed to an increase in the kinetics due to more energy and movement as an effect of an increment in temperature, which allows the dissociation of Bromacil from the nanoparticle.

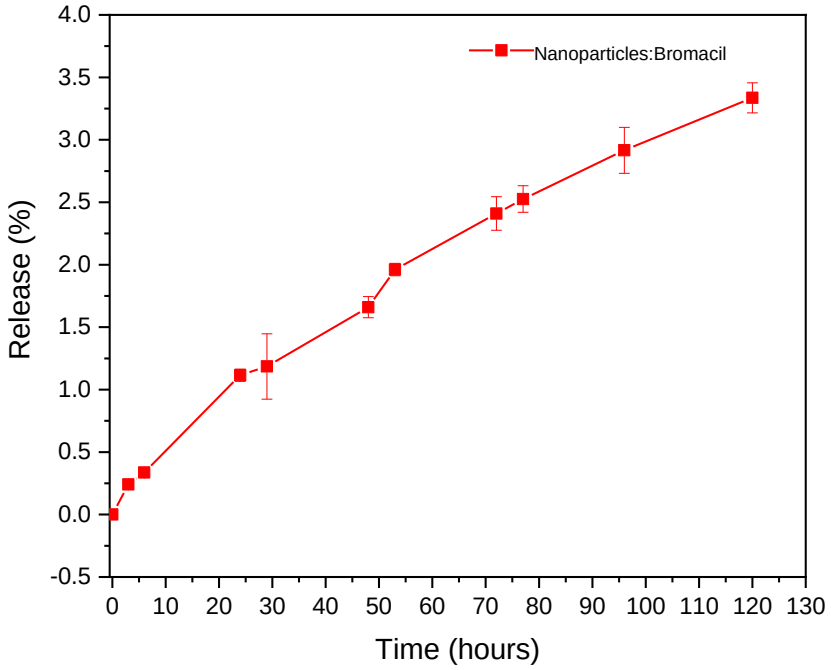

Figure 28 Release profiles in water of free Bromacil and Bromacil associated with the chitosan nanoparticles at 100ppm with pH 9.5-10 at 25°C

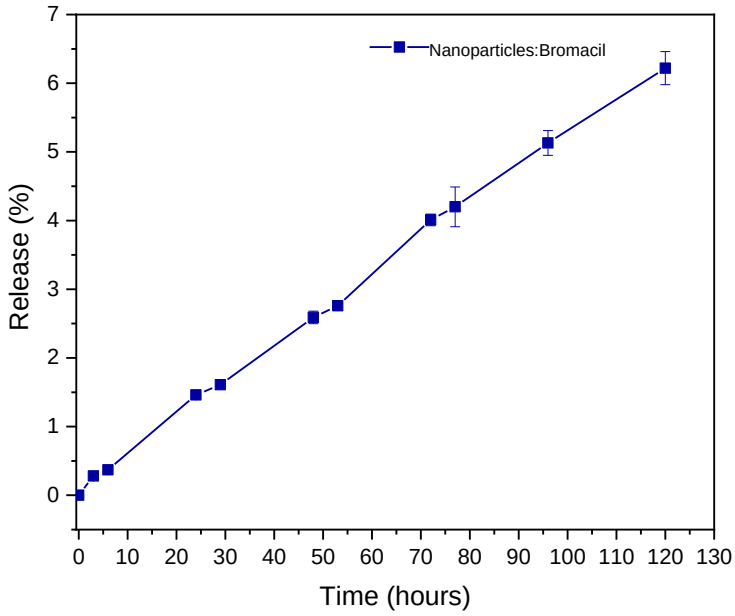

Figure 29 Release profiles in water of free Bromacil and Bromacil associated with the chitosan nanoparticles at 100ppm at 40 °C

5.2.2.3.3. Release mechanism

The release mechanism of herbicides from a polymeric nanoparticle is dependent of several factors, including desorption from the surface, diffusion through the pores or wall of the polymeric matrix and disintegration, dissolution or erosion of the polymeric structure [6]. In order to identify the type of mechanism involved from nanostructured systems, different research works reported the use of the mathematical model Korsmeyer-Peppas ([4], [6], [41], [43].

This model uses a simple exponential equation ($M_t / M_\infty = Kt^n$) to described the relationship between time and amount of the substance released. Its greatly useful when the mechanism involved is not well understood or when exist more than one mechanism. To calculate the release constant (*K*) and release exponent (*n*) a linear regression was used. The calculated values for these constant for each sample are presented in the Table 5. For all herbicide (except the one at 40°C) values of the release exponent (*n)* were in the range of $0.43 < n < 0.85$, indicating that the release mechanism involved in the release process was governed by anomalous behavior (Non-Fickian Release Kinetics). This means that the release mechanism of Bromacil might be a combination of diffusion and relaxation of the polymeric chains. The same mechanism was reported for the release of Paraquat from chitosan/alginate nanoparticles and from chitosan/trypolyphosphate nanoparticles [4], [6]. By contrast, the release exponent (*n)* of the herbicide exposed to 40 °C, were in the range of $n > 0.85$, which means that the mechanism exhibits a case Type II Transport (Non-Fickian Release Kinetics), controlled only by the relaxation of the polymer chains. Grillo et al., (2012) [41], identified this same mechanism for three herbicides (Ametryn, Atrazine, Simazine) released from a polymeric

poly(-caprolactone) nanocapsules. According to these results, two different diffusion mechanisms might be controlling the release of Bromacil herbicide.

Table 5 Values of the release constant (K), exponent (n), correlation coefficient (r) for the nanoparticles containing Bromacil with 80ppm, 100ppm,100ppm at pH 9.5-10 and 100ppm (40 °C)

Sample	Release constant *(K)*	Release exponent *(n)*	Correlation coefficient *(r)*
80 ppm	3.46×10^{-5} min^{-1}	0.81	0.971
100ppm	1.37×10^{-5} min^{-1}	0.85	0.998
100ppm (pH 9.5-10)	4.77×10^{-5} min^{-1}	0.74	0.996
100ppm (40 °C)	2.34×10^{-5} min^{-1}	0.88	0.993

5.2.3. Remarks

Bromacil herbicide were successfully incorporated in the chitosan nanoparticle. Nanoparticles with Bromacil obtained the greatest encapsulation efficiency (62%) by Method A. Zeta Potential analyses indicate that the nanoparticle with Bromacil suspension are moderately stable. Release experiments demonstrate a tendency of a slower release of the herbicide in comparison with the free compound. Initial concentration of the herbicide and changes in pH have no effect in the release Bromacil. The release mechanism of Bromacil is a combination of diffusion.

6. General Conclusions

Chitosan nanoparticles with size of approximately 18nm, were successfully synthetized by the polymerization of methacrilyc acid. Nanoparticles with different sizes were obtained varying the chitosan concentration. Structural and physicochemical characterization of the chitosan nanoparticle were achieved by FTIR and Zeta Potential analyses.

The herbicide Bromacil were incorporated to the chitosan nanoparticle by two methods presented. The synthesis method where the Bromacil were added at the time of preparation were the most effectively, obtaining a 62% of encapsulation efficiency. Hydrodynamic diameter indicated that the incorporation of Bromacil to the nanoparticle did not affect the size of the particle.

Release assays exhibits different percentages of release, with a maximum of a 6% of release in 120 hours (5 days). Kinetics profiles suggest that the release of Bromacil coming from nanoparticles was slower than the free herbicide. Initial concentration of the Bromacil and changing in pH did not affect the release profile of the herbicide. Expose the nanoparticles to temperatures caused an increase in the percentage release of the herbicide.

The results of the present study show that chitosan nanoparticles could be work as nanostructure controlled release system of herbicides. Further investigations are needed in order to maximize the release percentages and provide an efficiently control method of weeds.

7. References

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8. Appendix

8.1. Release Kinetics: Linearization using the Korsmeyer-Peppas mathematical model.

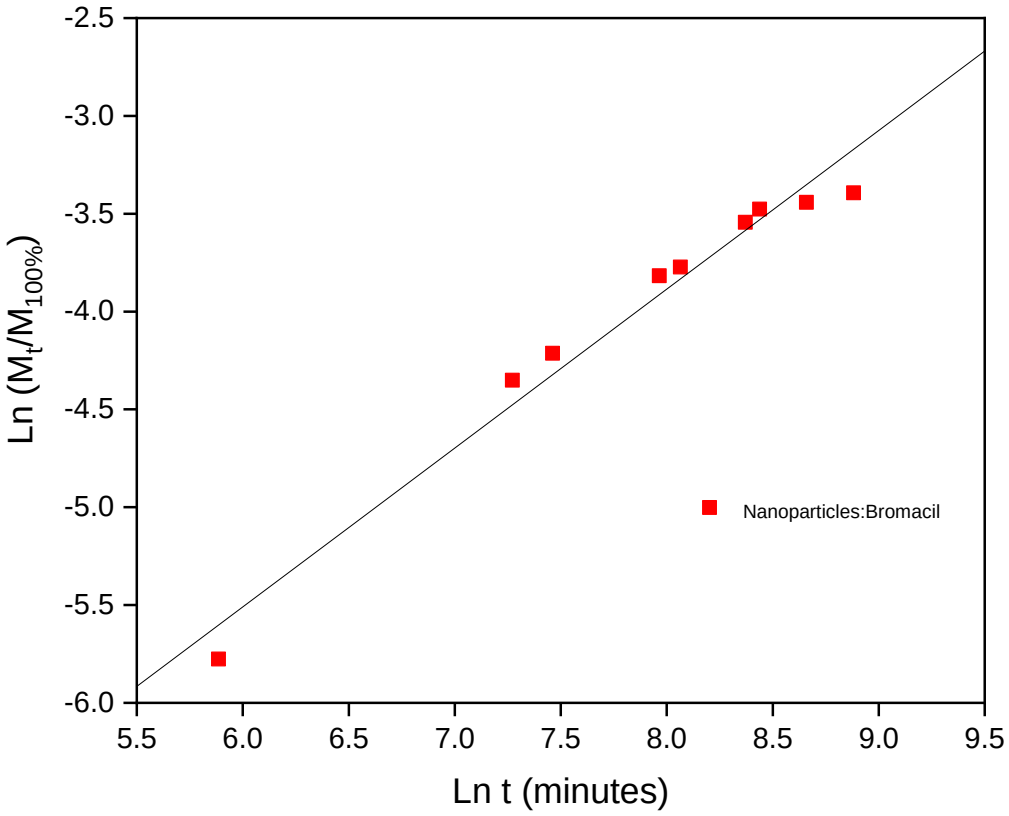

Figure 1: Linearization using the Korsmeyer-Peppas mathematical model for the herbicide Bromacil (80ppm) associated with the chitosan nanoparticle.

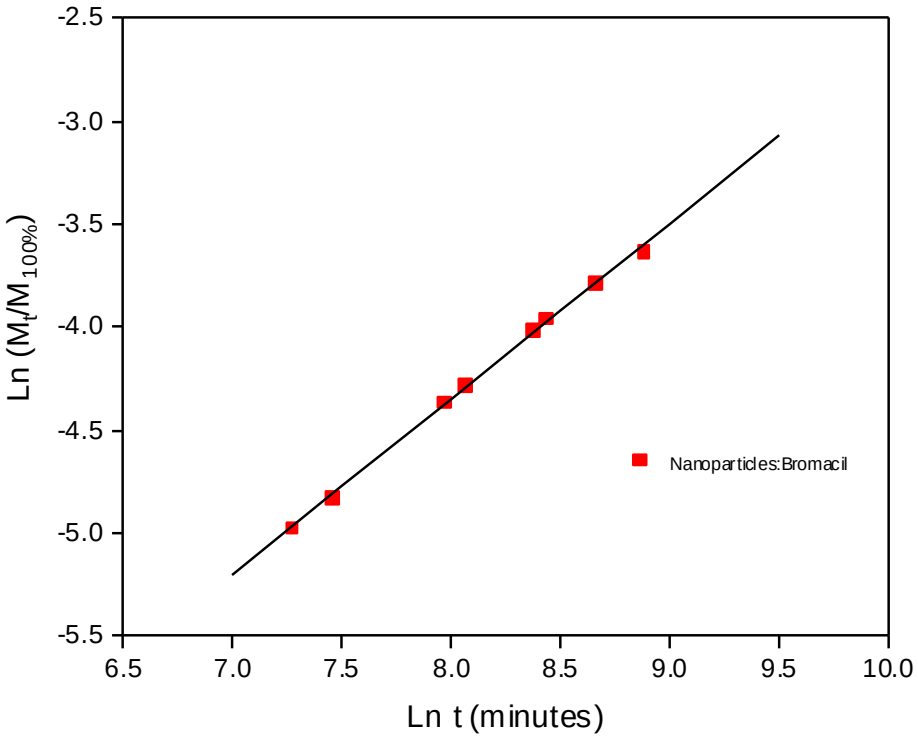

Figure 2: Linearization using the Korsmeyer-Peppas mathematical model for the herbicide Bromacil (100ppm) associated with the chitosan nanoparticle.

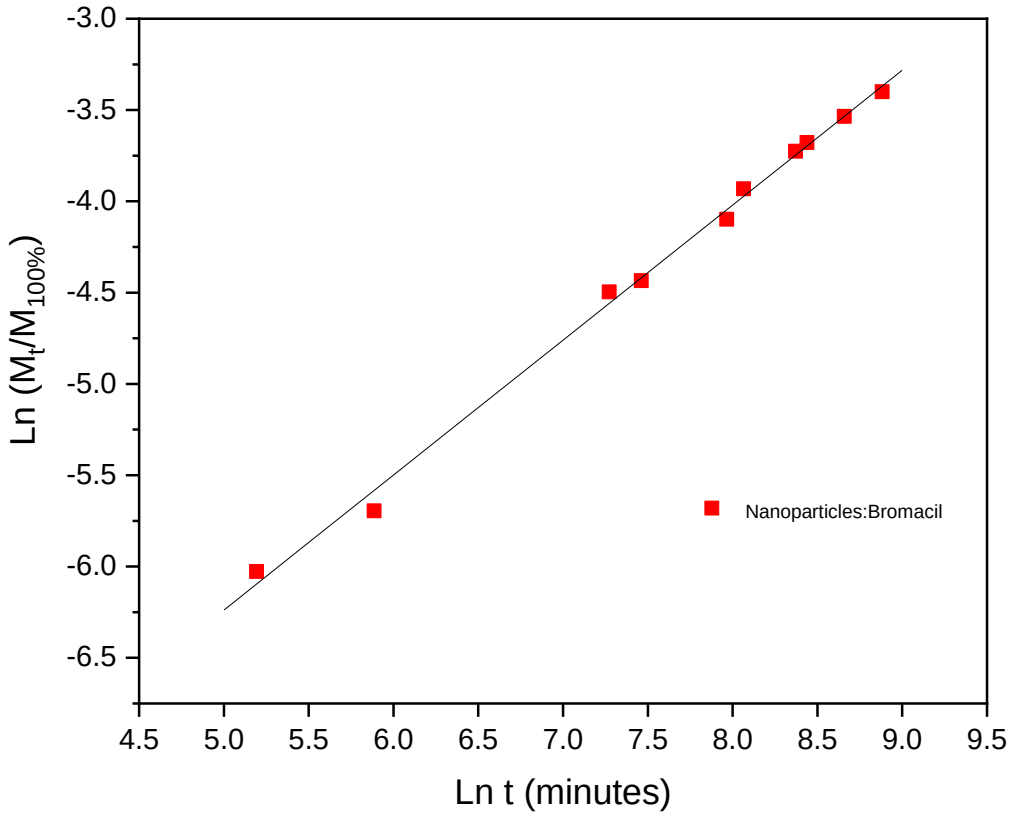

Figure 3: Linearization using the Korsmeyer-Peppas mathematical model for the herbicide Bromacil (100ppm, pH 9.5-10) associated with the chitosan nanoparticle.

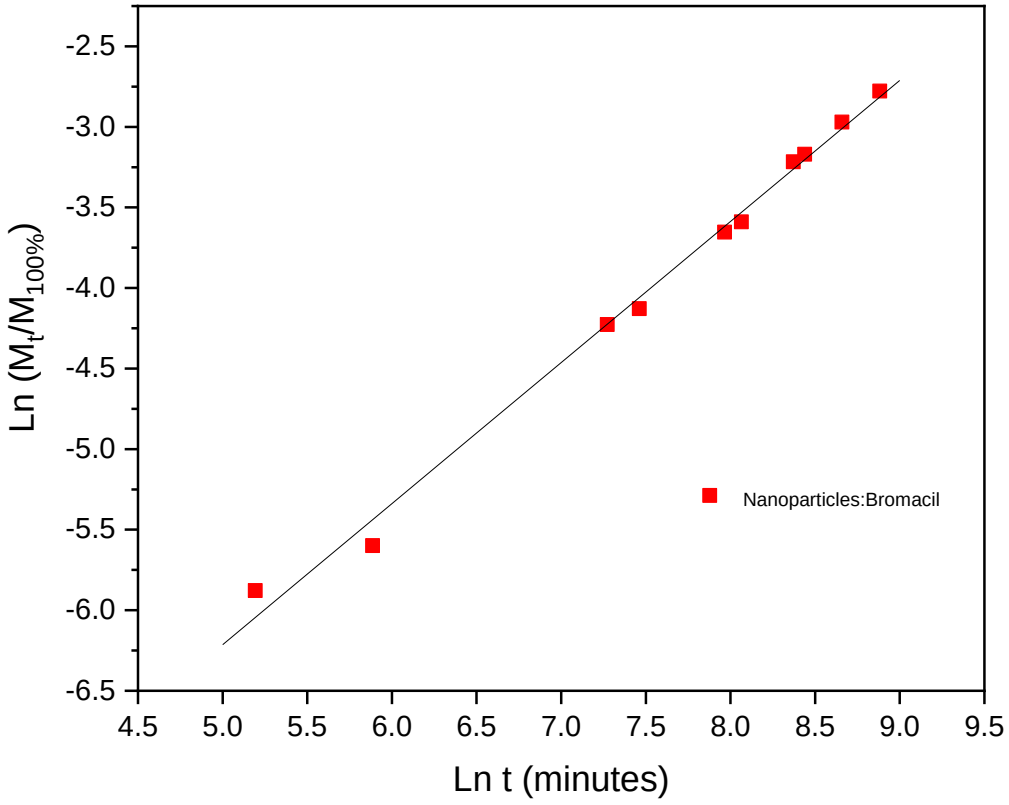

Figure 4: Linearization using the Korsmeyer-Peppas mathematical model for the herbicide Bromacil (100ppm, 40 °C) associated with the chitosan nanoparticle.