Preparation and characterization of loaded paraquat- polymeric chitosan/xantan/tripolyphosphate nanocapsules and evaluation for controlled release

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Abstract

Purpose Paraquat is an effective, non-selective, and fast-acting contact herbicide that is widely used. Its high solubility in water and adsorption in soil can easily poison the non-target organs. In this study, paraquat nano-hydrogels was synthesized using chitosan,

Methods Sodium tripolyphosphate and xanthan via ionic gelation method. After preparation the loaded paraquat formulations, to verify the morphology and analysis the functional groups on the formulation, SEM and FTIR analysis were used, respectively. In this work, stability of the formulation was measured in terms of size distribution, surface charge, and pH values. To determine the release kinetics, a dialysis bag was used. In addition, herbicidal activity of the prepared formulation was tested on corn bushes and wild mustard.

Results From the analysis, FT-IR spectra confirmed the hydrogel formation, and SEM images showed a dense structure in the synthesized hydrogel. According to the results of size distribution, surface charge, dispersion index and pH, it was proved that the prepared hydrogel was stable. The optimal values of chitosan, SPP, xanthan, and PQ were 0.3, 0.1, 0.15, and 20 mg, respectively. Based on the peppas equation, about 89.82% of the paraquat was released from the formulation with a paraquat loading of $89.1 \pm 4.6\%$.

Conclusions The effect of loaded paraquat formulations on mustard and corn plants showed that the herbicidal properties of the encapsulated paraquat were preserved. This study reveal that the loaded paraquat L-PQ is a stable formulation with less toxicity effects.

Keywords Paraquat · Encapsulation · Herbicide · Nanogel · Nanoparticle · Chitosan

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Introduction

Application of herbicides to protect the agricultural crops is inevitable. However, their use has a potential risk for humans, animals, and the environment [1, 2]. Therefore, a large amount of these chemicals can be found in food materials, drinking water, and soil. It is estimated that about 90% of herbicides do not reach to the intended site and finally enter to the surrounding environments (soil, water, and air) [3]. Quaternary ammonium salts (also known as quats), is a type of perilous herbicides for human and environment. From these compounds, paraquat (PQ) is commonly used to control weeds [4]. This chemical is a cation with a molecular weight of 186.3 g/mol, a contact herbicide, fast-acting, non-systemic and non-selective chemical [5]. Today, this chemical is one of the most-selling herbicides, and in this respect it ranked the second in more than 100 countries [5]. Paraquat is widely used to control the weeds in tobacco, cotton, rice, beans, coffee, sugar cane, apples, soybeans, grapes, and other products fields. In addition, it is used in planting management systems alone or in combination with other herbicides [6]. In plants, PQ affects electron transfer system and inhibits the conversion of nicotinamide adenine dinucleotide phosphate (NADP) to NADPH, ultimately results in plant destruction. Meanwhile, the produced reactive oxygen species (ROSs) can react with membrane lipids, leading to destruction of plant tissues and ultimately plant death [6]. PQ can also poison human via ingestion, inhalation, injection, or dermal contact. It can affect the lungs, heart, kidneys, adrenal glands, central nervous system, liver, muscle, and spleen. 30 mg/kg PQ compound (or 3 to 6 g of PQ ion), can result in death [5]. The United States Environmental Protection Agency (USEPA) has placed PQ in the list of priority chemical pollutants and highly toxic substances and regulated value of 3 µg/L as the maximum concentration level for this contaminant in drinking water. The European Union (EU) has not set any allowable levels for PQ in drinking water, but proposed a value of 0.1 µg/L for an individual pesticide and 0.5 µg/L for total pesticides [4]. However, different values of PQ have been reported in environment. For instance, in Spain in irrigated canals, rivers and wetlands the measured concentration of PQ was 3.95 µg/l and its concentrations in ground-water and surface waters of Thailand were 1.5–5.9 and 0.8–0.89 µg/L, respectively [7]. In addition, the residues of PQ have been reported in various crops such as olive (up to 10 mg/kg), onion, brussels sprouts (7.3 mg/kg), tomatoes (7.6 mg/kg), corn and sorghum products (69.7 mg/kg), and chick Pea 3.1 mg/kg. It can also remain in frozen food products up to 46 months [8]. One of the major problems caused by uncontrolled use of PQ is its high persistence in environment. It has been reported that PQ half-life was 16 months to more than 13 years. Although only a small amount of PQ is absorbed by soil, it has the potential of water contamination due to its high solubility (about 625 mg/L at 25°C) [9].

Commonly less than 0.1% of the pesticides reach to target organ, and the residue causes damage to non-target organs. Nowadays, the technology of controlled release in agriculture aims to encapsulate the pesticides with polymer and control their gradual release from the capsule to the weed. This technique can be a good way to reduce the pesticides toxicity and prevent environmental contamination. On the other hand, this method can minimize the loss of toxins against degradation, evaporation or washing. If pesticides are loaded into polymers, they are not released into the environment quickly, less substance will be consumed over a period of time than traditional method, and their toxicity will be lower [6]. Chitosan ($(C_6H_{11}O_4)_n$) is a natural organic linear biopolymer, derived from chitin deacetylation [10]. This polysaccharide due to many characteristics such as biocompatibility, good adhesion, high permeability, low cost, biodegradability, non-toxicity, non-cancerous, non-toxic, non-aggressive and other good properties is widely used as a carrier for effective release of active ingredient (drug) [11]. Ion gelation using sodium tripolyphosphate ($Na_3P_5O_{10}$) (TPP) has been more attractive than other cross-linkers due to relatively easy process control and no need of organic solvents. Xanthan ($C_{35}H_{49}O_{29}$) is the anionic extracellular heteropolysaccharide produced by fermentation via gram-negative bacteria, (*Xanthomonas* spp) [12]. The main structure of xanthan is quite similar to glucose. Among the microbial gum, xanthan due to its characteristics, such as plastic-like dispersion, high viscosity (even at low concentrations), stability, and compatibility with most metal salts, excellent and stable solubility in acidic and alkaline solutions, resistance to degradation at high temperatures and different pH levels has been of great interest [13]. Based on published papers, although encapsulation of substances has been mainly used for drugs delivery, but it has not been deeply studied for environmental purposes especially herbicides release control as a novel method. This study, aimed to prepare PQ loaded nano-hydrogels using chitosan, TPP and xanthan via ion gelation method and determine the herbicidal activity.

Materials and methods

Materials

All chemicals used in this study were of analytical grade. Materials needed in this study included Acetate (CH_3COOH), Chitosan, Sodium triphosphate ($Na_3P_3O_{10}$), Xanthan, Paraquat (PQ; 1,1-dimethyl-(4,4-bipyridium) dichloride) of commercial ($C_{12}H_{16}C_{12}N_2$) and technical ($C_{12}H_{14}N_2$), Hydrochloric acid (HCl), Liquid nitride (N_2), Estonitrile (C_2H_3N), Hexane sulfonic acid ($C_6H_{13}NaO_3S$), Disodium hydrogen monophosphate (Na_2HPO_4), Formalin (HCHO), Ketamine ($C_{13}H_{16}ClNO$), Xylazine ($C_{12}H_{16}N_2S$), Phosphoric acid (H_3PO_4), Paraffin (C_nH_{2n+2}), Pyrogallol

(C₆H₆O₃), Hydrogen peroxide (H₂O₂), Potassium Phosphate (K₂HPO₄), Ethanol (C₂H₆O), Coomassie Brilliant Blue (C₄₅H₄₄N₃NaO₇S₂), Mercury oxide (HgO), and Potassium Alum (KAl(SO₄)₂). Total chemicals required was prepared from Merck, Sigma-Aldrich companies.

Preparation of chitosan/xanthan-based paraquat loaded nano-gel

Optimization of chitosan

In this study the nanoparticles were synthesized via ionic gelification method that widely has been used in previous published works [14–16]. To optimize chitosan, various amounts (0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.6%, and 0.8%) of chitosan were gradually added to acidic solution (15 mL, pH = 4.7) and was placed under intense agitation for 2 h. Then, the solution was filtered with filter paper (No. 3) and added PQ (20 mg) to each container. In the final step, sodium TPP solution (15 mL, 0.02%) and xanthan solution (2 mL, 0.25%) (pH = 2) was added.

Optimization of sodium tripolyphosphate

To optimize sodium TPP, after adding paraquat (20 mg) to chitosan (0.3%), (15 mL) of different concentrations of sodium TPP solution [16] 0.1%, 0.2%, 0.3%, 0.4%, 0.6%, and 0.8%) were added drop wise to the solutions. Finally, xanthan solution (2 mL, 0.25%, pH = 2) was added to each container.

Optimization of xanthan

To optimize xanthan, after adding paraquat (20 mg) to chitosan (0.3%), sodium TPP solution (15 mL, 0.1%) was added to the solutions. Finally, various concentrations of xanthan (0.05%, 0.083%, 0.11%, 0.15%, 0.18%, 0.21%, and 0.25%) were added drop wise to the solutions.

Optimization of Paraquat herbicide

To optimize paraquat, 0.5 mL of different concentrations of paraquat (5, 10, 15 and 20 mg) were added to 0.3% chitosan solution containers, then, 15 mL of TPP solution (0.1%) and after that, 2 mL xanthan solution (0.15%) was added (pH = 2) to above containers. During the process, all solutions were added dropwise and after adding each substance, the solutions were subjected to intense agitation for 30 min. To load PQ, a solution containing chitosan (0.3%) in acetic acid (0.2%)(pH = 4.7) was prepared and placed under constant stirring for 2 h. [16] To remove insoluble residuals, the solution was filtered using a filter paper (No. 3). After complete dispersing of chitosan, 20 mg PQ was added to the solution. For

complete dispersal of PQ in chitosan solution, the sample was subjected to continuous stirring for 30 min. Then, TPP solution (0.1%) was added drop wise to the solution to create cross-linking with chitosan and forming a hydrogel network. After the formation of the hydrogel, 2 mL of xanthan solution 0.15% (prepared in HCl (2 M) at 90 °C) was added to the formulation containing PQ. After completion of the synthesis process, the hydrogel was dried using liquid nitrogen and dried with freezer dryer [15]. To determine the encapsulation efficiency, 0.20 g of freeze dried hydrogel was poured into the test tube and after adding 4 mL of hydrochloric acid 2 M in the test tube for 30 min in an ultrasonic apparatus. Finally, the filtrate content was distilled into 10 mL volumetric distilled water and injected into the HPLC apparatus (Instrument Model: Varian VISTA-MPX) to determine the amount of loaded paraquat. The amount of paraquat loaded in the final formulation was calculated according to the following equation (Eq. 1) [17]:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{loaded paraquat (mg)}}{\text{theoretical loaded paraquat (mg)}} \times 100 \quad (1)$$

Formulation stability evaluation and characterization

The stability of the synthesized formulation was measured in terms of diameter, zeta potential, dispersion index, and pH for 60 days [14]. To measure the diameter, zeta potential and dispersion index in the prepared nanoparticles, a Nano Zeta analyzer (ZEN model 3600 by Malvern England) was used. The morphological characteristics of the dried nano-gels were determined by scanning electron microscopy (SEM) (SIGMA VP-500 model, ZEISS Company). Surface functional groups analysis of the prepared nanogels were conducted using a FTIR device (Tensor 27, Bruker Inc. Germany). To evaluate the pH of the manufactured hydrogels, the pH of the unloaded and the loaded formulation with PQ were measured at various time intervals at 25 °C. Their pH values were measured over 0, 1, 2, 7, 14, 30, 45, and 60 days intervals.

PQ release evaluation in vitro experiment

Evaluation of the kinetics of nanoparticle release in this field is of great importance. In this study, dynamic dialysis was used for determining the release kinetics of PQ release [14, 18]. To evaluate the release rate of PQ from the formulation, 0.36 mg of the sample was poured into the dialysis bag and tightly closed. Then, dialysis bag containing PQ donor phase (pH = 4.5) was completely immersed in a beaker containing 250 mL of the receptor phase (pH = 9.2). To measure the amount of PQ released from the formulation, 0.5 mL of the receptor

solution were removed at the known period of time (30, 45, 75, 120, 210, 240, and 270 min) at room temperature and immediately replaced with 0.5 mL of fresh solution. The concentration of paraquat released from the dialysis bag was read by a spectrophotometer [19]. The release mechanism was studied using the semi empirical Korsmeyer–Peppas model. In general, this equation (Eq. 2) is used to interpret and describe the diffusion of different materials at a time when the dominant mechanism is not known or when it is combined with two independent processes [6, 20, 21]:

$$\frac{M_t}{M_\infty} = Kt^n + b \quad (2)$$

Where, M_t is the amount of paraquat released from the nano gel at time t , and M_∞ is the amount of PQ released at the infinite time. In this equation, the K is kinetic constant depends on the structural and geometric properties of the process, and n exponent indicates the release mechanism. According to n value the release mechanism can be defined, if $n < 0.43$, the release mechanism is purely by diffusion (Fickian release); if $n > 0.85$, the dominant release mechanism of the diffusion, which is the case of the case II, with polymer swelling (polymer matrix relaxation); if $0.43 < n < 0.85$, the mechanism obeyed anomalous transport indicating the combination mechanism including a combination of emission mechanisms and II mode [6, 20, 21].

Herbicidal activity of the prepared formulation

To evaluate the activity of the herbicide, the loaded PQ formulation, corn seeds (*Z. myze* as non-target plants) and mustard (*Brassica* spp. as target plant) were planted in identical plastic pots with four replicates. The number of planted seeds per pot was 10, and the soil/fertilizer/sand ratio was 2/1/1. The irrigation of the pots was done every day for 20 days. After this time, different groups control (DW), unloaded paraquat formulation (U-PQ), free technical paraquat (T-PQ), commercial paraquat (C-PQ), the formulation paraquat at concentration of 500 g per hectare of paraquat (L-PQ-500 g/ha), the formulated PQ with a concentration of 400 g per ha (L-PQ –400 g/ha) of paraquat and the formulated toxin group with a concentration of 300 g per ha of paraquat (L-PQ –400 g/ha) (on weed and corn plants) were affected. The control group was only received distilled water. The amount of paraquat sprayed in each pot was proportional to the amount used near to real agricultural conditions (equivalent to 2.5 L/ha). After 48 h, 5 plants were randomly selected from each pot and completely dried in an oven at 70 °C for 24 h and finally the dried weight was recorded [14].

Results and discussion

Selection the optimal values of chitosan, sodium tripolyphosphate, xanthan, and PQ for the formulation

To select the optimal values of different variables, in each step, one of the variables was changed and the rest were kept constant. According to Fig. 1 (a, b, c, and d), the optimal values of chitosan, SPP, xanthan, and PQ were 0.3, 0.1, 0.15, and 20 mg, respectively. These values were used as optimum values for the formulation preparation and the experiments. The efficiency of loaded PQ in the final formulation was $89.1 \pm 4.6\%$. In another study conducted by Niculina Popa et al. (producing chitosan-xanthan-based hydrogels to control the release of theophylline), the amount of loaded theophylline was 88.6% [22]. Sandeep Kumar et al. reported 62% of acetamiprid encapsulation efficiency using alginate/chitosan nano-gel [23]. While Mariana de santos et al. reported 74.2% of Paraquat-loaded on alginate-chitosan nanoparticles [6]. Grillo et al. showed a 62.6% of encapsulation of Chitosan/tripolyphosphate nanoparticles loaded with paraquat [14]. The reasons for differences in encapsulation efficiencies can be due to solubility of loaded materials (e.g. pesticides) in polymers and the surface charges. In addition, the amount of applied materials, experiment methodology, type, and properties of the materials are all affect the encapsulation efficiency [6, 23]. Therefore, in the present study, the high efficiency of paraquat encapsulation (89.1%) can be due to the use of SPP as an effective cross-linking for xanthan, which has the ability to produce high surface charges [14] and conducting the optimization process in this study to choose the best concentration of the main formulation material. It should be noted that by selecting the appropriate value of each ingredient, the efficiency in the next step was increased as shown in Fig. 2. This high value of loading indicated the proper interaction between synthesized nano hydrogel components due to formation of an electrostatic bond between anionic functional groups of SPP and cationic functional groups in chitosan, which results a porous structure hydrogel and eventually PQ loading in the pores.

Stability of the formulation

The stability evaluation was carried out for loaded PQ (L-PQ) and unloaded PQ (U-PQ) formulations in terms of hydrodynamic diameter (nm), zeta potential (mV), poly dispersion index (PDI), and pH over a 60-day period. The results are presented in Table 1. According to Table 1, a slight increase was observed between the initial diameter and the diameter after 60 days for both U-PQ and L-PQ. The reason for this increase in the size of the nano-gel may be due to the reaction of chitosan amine groups with negative charge on TPP or

Fig. 1 Selection of optimum amount of chitosan (a), sodium tripolyphosphate (TPP) (b), xanthan (c), and (d) PQ for formulation preparation

absorption of moisture by xanthan [15]. The advantages of nano-gels are their small size, which results in increase in the surface to volume ratio, induces systemic activity, and increase the nanoparticle stability [11, 24, 25]. Nano-gel size is significantly affected by TPP and chitosan concentrations, degree of chitosan deacetylation (lower degree of deacetylation results in, larger particle sizes with low ability of keeping the active materials), the mixing speed during the process, the acetic acid concentration (used to dissolve chitosan), and the temperature (at which cross-linking are created) [11, 15].

Zeta potential is another indicator of hydrogels stability. Nanoparticles with a zeta potential of more than ± 30 mV remain stable in the suspension because repulsion forces between particles can prevent coagulation and clumping [14, 23]. The value of zeta potential shows that the fabricated

nanoparticles were stable. In this study, zeta potential was positive due to cationic polymers of chitosan. After 60 days, zeta potential increased in L-PQ formulation and non-unloaded formulation with paraquat, which could indicate that with the passage of time, PQ released via erosion mechanism. Therefore, the presence of positive surface charges of chitosan (NH_2^+) and PQ increases the zeta potential in this formulation [15, 26]. The increase in positive charges increases the repulsion forces and reduces the auto coagulating of the particles [27]. On the other hand, the zeta potential of the L-PQ formulation was higher than that of the U-PQ, indicating that PQ, after loading in the hydrogel, increases the zeta potential and stability. The dispersion index is another parameter for assessing the hydrogels stability [28]. A value of PDI < 0.5 is desirable [25, 29]. The low amount of PDI formulations makes it easier to attach particles together and reduce auto clumping and ultimately increase the stability of the nano-gel [29]. This index has not changed significantly over time in the unloaded formulation with PQ, while the L-PQ formulations have shown a slight increase. The reason for this increase may be due to the reaction of PQ with the anionic

Fig. 2. pH value of the loaded and unloaded paraquat formulation.

Table 1 Sustainability Indicators for loaded PQ (L-PQ) formulation and unloaded PQ (U-PQ) formulation

	First day		After 60 days	
	U-PQ	L-PQ	U-PQ	L-PQ
Diameter(nm)	150.8	227.9	170.9	403
Zeta potential (mv)	12.5	20.9	23.9	32.2
Dispersion index	0.279	0.316	0.214	0.408

groups in TPP [14]. The finding of this study is closely in line with the results reported by other researchers working with the same materials for nano-gel synthesis [14]. Another stability indicator is pH values changes measurement. Bipyridinium cations such as PQ are one of the most widely known group of the most well-known ionized herbicides, so evaluation the role of pH on their performance is essential. To evaluate the pH of the fabricated hydrogels, the pH of the unloaded formulation with PQ and the loaded PQ were measured at various time intervals over 0, 1, 2, 7, 14, 30, 45, and 60 days intervals at 25°C. The results of pH values changes is presented in Fig. 2. According the Fig. 2, the pH of the synthesized nano-gels (L-PQ and U-PQ) stay nearly constant up to 30 days but after that goes into alkaline range over 60 days. Griloo et al. reported a decrease in pH values over time at initial acidic pH (about 5) [14]. It seems that the media range of pH for stability evaluation is an important factor. So, practically point pH media in filed soil can be an important issue. However, in present study, the pH range media was neutral (around 7). The increase of pH (after 60 days) can be due to presence of TPP neutralize glucosamine over time or an alternation of the charges between the negative charges (TPP and xanthan) and positive charges in chitosan. It has been reported that isoelectric point of chitosan–TPP complex is around 7.5. The alternation of negative and positive charges can occur at basic pH that the TPP functional groups, uncharged the glucosamine groups of chitosan [30]. In general, the results of physicochemical analysis showed that the synthesized nano-gels in terms of the studied parameters are stable and can be stored for a period of 60 days at room temperature.

Surface morphology and surface functional groups of the prepared formulations

The morphology of the formulated herbicide was evaluated using a scanning electron microscopy (SEM) equipment was used. The SEM image of unloaded formulation with PQ is presented in Fig. 3. SEM images show the porous structure of U-PQ formulation with a fully dense matrix of L-PQ formulations. The reason of the porosity and cavity formation in this hydrogel is the electro-static cross-linking of sodium TPP with amino groups in chitosan. FTIR spectrophotometry was used to verify the synthesis of the product and to confirm the presence of functional groups on the synthesized formulation [31, 32]. The results of FTIR spectra are presented in Fig. 4.

Based on chitosan FTIR spectrum (Fig. 4 a), the strong peak at 1611 cm^{-1} assigned to bonds of (N-H) amide II, (C=O) (acetamide amide I), and (C-O) amide I. The weak peak at 2877 cm^{-1} corresponds to C-H band and the broad band at 3345 cm^{-1} assigns to NH_2 , N-H, and O-H bands and the peak at 1160 cm^{-1} assigns to C-O bond [18, 22, 33]. For FTIR spectrum of TPP (Fig. 4b), depicts the strong band at 1216 cm^{-1} assigns to P=O and the broad

band at 1165 cm^{-1} attributed to P-O (polyhedron groups) [33]. For Xantan (Fig. 4c), a strong band at 640 cm^{-1} is attributed to -COO bond and a weak peak at 1415 cm^{-1} is assigned to C=O stretching vibration ban symmetric carboxylate ($-\text{COO}^-$), while the strong -C=O stretching vibration ban asymmetric carboxylate ($-\text{COO}^-$) appeared at 1611 cm^{-1} . The broad band O-H (hydroxyl group) 3347 cm^{-1} , the broad band also associated with the assigned to C-O (type I alcohols) 1092 cm^{-1} groups. [22]. From the FTIR of PQ (Fig. 4d), distinct strong peaks located at 2926 cm^{-1} , assign to C-H bond, and a strong bond relation to C-N and C=N seen at 1638 cm^{-1} . The peak at 1638 cm^{-1} is assigned to the stretching vibration of C=N/C-N in the PQ molecules while a weak bond at around 1510 cm^{-1} corresponds to the vibration of $\text{H}_3\text{C}-\text{N}^+$ bonds and the strong stretching band at 1345 cm^{-1} reflects the N-H (aromatic amines) and that of 810 cm^{-1} reflects the strong reformation aromatic ring of PQ molecule [6, 16, 34]. In the IR spectrum of U-PQ (Fig. 4 (e)), there were broad and strong absorption peaks at wavelengths of 1154 cm^{-1} and 1215 cm^{-1} , indicating the presence of P-O and P-O groups corresponding to TPP. The presence of this peak represents the cross-linking created by TPP, and the absorption peaks at 624 cm^{-1} , 3407 cm^{-1} , 1618 cm^{-1} , and 1416 cm^{-1} also confirms the presence of -COO, OH, -C=O and symmetric and asymmetric starching vibration of -COO-, respectively, related to xanthan. The absorption peaks at 1154 cm^{-1} , 1618 cm^{-1} , and 3407 cm^{-1} indicates the presence of CO, amide II (NH), C=O (acetamide amide A) and (CO) amide I, NH_2 and N-H in chitosan which confirmed the formation of hydrogel based on xanthan and chitosan. IR spectrum of the L-PQ (Fig. 4f), broad and weak absorption peaks at 1154 cm^{-1} , indicating the presence of P-O groups corresponding to sodium TPP. The presence of this peak in the corresponding spectrum represents the cross-linking connections created by TPP. In addition to the presence of functional groups related to chitosan and xanthan in the L-PQ formulation, there was a strong absorption peak at 1639 cm^{-1} relating to PQ indicates the loading of PQ in the formulation. The intensity of the absorption peak assigned to -COO at 1625 cm^{-1} increased in the IR spectrum of the encapsulated paraquat, and the intensity of the absorption bands of C-O and P-O at 1154 cm^{-1} also increased. According to FTIR spectra analysis, it can be confirmed that presence of PQ in the synthesized L-PQ formulation.

PQ release from the formulation

The trend of PQ release from the polymer nano-gel matrix was investigated in comparison with free PQ. In laboratory conditions, at about 270 min, about 89.82% of PQ was released

Fig. 3 The SEM image of (a) paraquat unloaded formulation (U-PQ) (b) paraquat loaded formulation with (L-PQ)

from the formulation (Fig. 5a and b). It has been stated that the controlled release of active ingredient from chitosan-based particles strongly depends on several factors including surface morphology, particle size, density, porosity, cross-linking agent type, encapsulation efficiency, physicochemical

characteristics, and amount of the loaded substance [3, 11]. Grillo et al. reported that the release rate of 70% at 350 min, from PQ encapsulated with chitosan/tripolyphosphate nanogel [14]. In the synthesized formulation, according to the peppas equation (Eq. 2), n was 0.91. Therefore, the

Fig. 4. the FTIR spectrum of chitosan (a), TPP (b), Xantan (c), PQ (d), U-PQ formulation (e), and L-PQ formulation (f).

Fig. 5 Accumulation diagram of the release of paraquat from hydrogel (a) and its paraquat release from hydrogel using model (b) over 270 min

synthesized polymer used erosion mechanism for its release. The same mechanism was obtained by Niculina Popa et al., [22].

Evaluation of the herbicidal activity of the formulation

The experiments showed that the encapsulated PQ is a stable formulation that helps the reduction the toxicity of this herbicide. The herbicidal activity of the encapsulated paraquat was investigated using two types of monocot (*Z. mays*) and dinocot (*Brassica* spp) plants. Both, free and encapsulated paraquat forms, were sprayed on both plants and induced necrosis and fading of the leaves within 48 h. In this study, according to Fig. 6a and b, the dried weight of corn plants in a group exposed to encapsulate PQ was more than that of free and commercial PQ.

It was revealed that both, T-PQ and L-PQ, induced necrosis and fading of the leaves within 48 h. In this study, dry weight of corn plants was higher as exposed to L-PQ compared to the plants exposed to T-PQ and C-PQ. Therefore, from the assays, the prepared formulation has less damage to corn plant. On the other hand, the dry weight of the mustard plants in the group exposed to L-PQ is less than the plants exposed to T-PQ and C-PQ. Therefore, it can be concluded that the prepared formulation has more destructive effect on weeds. The resistance of

Fig. 6 Herbicidal activity of paraquat in maize (*Zea mays*) and mustard (*Brassica* sp.) [The Mean $\pm$ SD values of dried plants after cultivation (48 h), treatment and drying. DW = the group that received distilled water as control group, U-PQ = the formulation without loaded paraquat, L-PQ (500 g/ha PQ), L-PQ (400 g/ha PQ) and L-PQ (300 g/ha PQ) are the paraquat loaded formulations at different concentration for plant cultivation (g/ha), T-PQ = the group that received technical paraquat, C-PQ = the group that received commercial paraquat, * Significant difference between DW ($P_{\text{value}} < 0.05$), **Significant difference with DW ($P_{\text{value}} < 0.01$), ***significant difference with the DW ($0/001 > P_{\text{value}}$), # significant difference with the U-PQ ($P_{\text{value}} < 0.05$), ## significant difference with U-PQ ($P_{\text{value}} < 0.01$), ### Significant difference with the U-PQ ($0/001 > P_{\text{value}}$), \$ significant difference with PQ ($P_{\text{value}} < 0.05$), \$\$ significant difference with PQ ($P_{\text{value}} < 0.01$), \$\$\$ significant difference with PQ ($0/001 > P_{\text{value}}$), + significant difference with C- PQ ($P_{\text{value}} < 0.05$)

corn bushes to formulations is probably due to the presence of enzymes such as P-450S glutathione-S-transferase P450 in corn bushes. This enzyme can neutralize the radicals produced by the toxin and cause corn bushes to resist paraquat [35]. Therefore, the destructive effect of paraquat on mustard plants was higher than that of corn and results in a faster necrosis of mustard plants. So, corn bushes were less damaged than mustard plants. Free-encapsulated paraquat caused faster necrosis of the plants, probably due to the attaching the nano-gels to the plant leaf and the increase of the effective and available concentration of paraquat for the plant. Other works showed that chitosan could increase the adhesion of nano-gels to the surface and thus increase the effect of the active agent on the plant [14, 35].

In the case of corn, as shown in Fig. 6, the use of an empty capsule or only a significant difference was not shown in

comparison to the control group. The use of PQ (commercial or technical) or encapsulated type with all three concentrations has an effect on maize and decreases its growth ($P_{\text{value}} < 0.05$).

However, the concentrations of the encapsulated species do not show a proportional trend the effect of paraquat on this plant, corn. But compared to the free expression of 0 and more) and encapsulated, the free type is more effective. As can be seen, freedom of both (both) has a significant effect on the weight loss of corn compared to the encapsulated type without PQ, which in this regard has a more technical effect. It appears that the presence of agents in the capsule creates a protective condition on the corn. Comparing different concentrations of capsules containing paraquat and type of paraquat (both), there was a significant difference ($P_{\text{value}} < 0.01$). In the case of mustard weed, it was found that in comparison with the control group, there was a significant difference ($P_{\text{value}} < 0.01$). In this regard, the encapsulated species with a concentration of 400 g per hectare was more effective than the other two concentrations, and in the case of maize, the same concentration was more effective. In comparison with the commercially encapsulated group with concentrations of 300 and 400 g/ha, a significant difference was found at $P_{\text{value}} < 0.05$. Compared to the encapsulated groups containing paraquat (all concentrations), there was no significant difference between the commercial and the technical grade of the product. This proves that the encapsulated paraquat leaves the same effect that the release forms on the weed, and their effect is preserved. According to the results of this section it was determined that the encapsulated paraquat had a lower effect on the corn plant relative to the herbicide type and on the other hand, the effect on the weed was similar to that of the free type. Herbicide encapsulation does not have a negative effect on the herbicidal effect.

Conclusion

In this study the nanoparticles were synthesized via ionic gelification procedure. Dynamic dialysis was used for determining the release kinetics of PQ release. The release mechanism was studied using the semi empirical Korsmeyer–Peppas model. To evaluate the activity of the herbicide, the loaded PQ formulation, corn seeds and mustard were planted in identical plastic pots with four replicates. The activity of the superoxide dismutase enzyme (SOD) was conducted according to McCord and Fridovich methods. The stability evaluation was carried out for loaded PQ (L-PQ) and unloaded PQ (U-PQ) formulations in terms of hydrodynamic diameter (nm), zeta potential (mV), poly dispersion index (PDI), and pH over a 60-day period. The efficiency of loaded PQ in the final formulation was $89.1 \pm 4.6\%$. The amount of applied materials, experiment methodology, type, and properties of the materials are all affect the encapsulation efficiency. The

advantages of nano-gels are their small size, which results in increase in the surface to volume ratio, induces systemic activity, and increase the nanoparticle stability. The morphology of the formulated herbicide was evaluated using a scanning electron microscopy (SEM) equipment was used. SEM images show the porous structure of U-PQ formulation with a fully dense matrix of L-PQ formulations. This study reveal that the loaded paraquat L-PQ is a stable formulation with less toxicity effects. The herbicidal activity of the encapsulated paraquat was investigated using two types of monocot (*Z. mays*) and dinocot (*Brassica* spp) plants. The dried weight of corn plants in a group exposed to encapsulate PQ was more than that of free and commercial PQ.

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Author's contribution All authors distributed, collected and read the dosimeters, analyzed, wrote the text, reviewed and approved the final manuscript.

Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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