Preparation of Copper-loaded Microcapsule Formulations

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Summary

Novel copper-loaded chitosan or chitosan/alginate based microcapsules formulations have been presented. It was shown that prolonged release of copper from microcapsules accompanied with possible prolonged presence of copper on leaves is useful in crop protection.

Key words

alginate, chitosan, copper, crop protection, microcapsule

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Introduction

Microcapsulation is an efficient method for controlled and/or prolonged release of an active agent (Green et al,. 2007). It is very useful if some environmental concerns about active ingredients demands reduced levels of chemicals to be used, as could be in case of copper usage (Hadwiger et al., 2006). Up till now, various crop protecting agents were encapsulated, e.g. pesticides, insecticides, fungicides (Fernandez-Urrusuno et al., 2000; Boehm et al., 2000; Tsuji et al., 2001; Boehm et al., 2003; Frederiksen et al., 2003; Hirech et al., 2003; El Bahri et al., 2007; Flores Céspedes et al., 2007; Kimoto et al., 2007; Sopeña et al., 2007; Guan et al., 2008; Li et al., 2008). Natural polyelectrolytes, such as chitosan and alginate, are known microcapsules forming polymers. They are common and abundant in nature, nontoxic and therefore suitable for microencapsulation.

Chitosan is natural polysaccharide derived by deacetylation of chitin consisting of varying amounts of β (1-4)-linked 2-acetoamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose and therefore it is a cationic polyelectrolyte. Chitosan is hydrophilic, biocompatible, biodegradable and bioadhesive polymer useful in many fields – from food industry and medicine to crop protection (Bautista-Baños et al., 2006). In addition, chitosan is nontoxic agent with antibacterial (Qi et al., 2004) and antifungal (Muñoz et al., 2008) properties and it is capable of inducing a defence mechanism in plant tissues (Aziz et al., 2006). Grapevine (Giannakis et al., 1998; Aziz et al., 2006), grapes and tomato (Muñoz et al., 2008), potato (Hadwiger et al., 2006) and celery (Bell et al., 1998) are just few examples of crops been successfully treated with chitosan alone or in combination with other active agents. Alginate is a linear, anionic polyelectrolyte, binary copolymer that consist of (1-4)-linked β-D-mannuronic acid and α-L-guluronic acid residues. Therefore, a combination of alginate (polyanion) and chitosan (polycation) forms a complex polyelectrolyte system suitable for microencapsulation (Saether et al., 2008).

This paper presents methods for preparing different copper-loaded, chitosan and chitosan/alginate macro- and microcapsules formulations useful in crop protection.

2. Materials and methods

Materials

Low molecular weight chitosan (Mr 150 000) was purchased from Fluka (Switzerland). Sodium alginate (viscosity 20 000 – 40 000 cps) was purchased from Sigma Aldrich (USA). A commercially available product (Cu-product) containing copper (II)-sulphate (35% Cu) was used as a copper donating substance. All other chemicals were of analytical grade and purchased from Kemika (Croatia).

Preparations

a) Preparation of copper-loaded, chitosan-based microcapsules

Copper-loaded, chitosan-based microcapsules were prepared by spray-drying of Cu-product dispersion in a chitosan solution using Büchi 190 mini spray-drier (Flawil, Switzerland) with a standard 0.5 mm nozzle. The liquid was fed to the nozzle with peristaltic pump, atomized by the force of the compressed air and blown together with a hot air to the chamber where the solvent in the droplets was evaporated. The dry product was then collected in a collection bottle. The drying conditions were as follows: spray flow rate of 0.25 L/h, compressed air flow rate of 700 NL/h, inlet air temperature of 135°C and outlet air temperature of 85°C.

Chitosan was solubilized in 0.5% acetic acid solution at 1% (*w*/*v*) concentration. Cu-product was dispersed in distilled water (0.1%, *w*/*v*). In order to prepare Cu-product dispersion in chitosan solution, chitosan solution and Cu-product dispersion were mixed in a 1:1 (*v*/*v*) ratio and subjected to spray drying under process conditions described above. The chitosan/Cu-product weight ratio in the spray-dried system was of 10:1.

b) Preparation of copper-loaded, alginate/chitosan mixed capsules

Copper-loaded, alginate/chitosan mixed capsules were prepared by spontaneous formation of alginate/chitosan polyelectrolyte complexes, as described below.

Chitosan was solubilized in 0.5% acetic acid solution at 0.15% (*w*/*v*) concentration. The pH of the chitosan solution was raised to pH 6.0 with NaOH 10 M. Sodium alginate was solubilised in water at 1% (*w*/*v*) concentration. Cu-product (0.6%, *w*/*w*) was dispersed in sodium alginate solution and stirred at 20°C. This mixture was injected dropwise (syringe inner diameter of 0.40 mm) into a chitosan solution which contained $CaCl_2$ (50 mM). The weight ratio between the alginate/Cu-product dispersion and the chitosan/$CaCl_2$ solution was 1:4. The capsules were cured in the solution for 1 hour at 20°C. Afterwards the solution was filtered and washed thoroughly in deionised water. The wet capsules were stored in deionised water at 4°C until further studies. A number of the capsules were freeze-dried (Freeze dryer Alpha 1-4, Christ, Germany) for 24 h and stored at 4°C until further studies.

c) Preparation of copper-loaded, alginate/chitosan micro-capsules viscous dispersion

Copper-loaded, alginate/chitosan micro-capsules viscous dispersions were prepared by mixing chitosan solution with Cu-product/alginate dispersion, as described below.

Chitosan was solubilized in 0.5% acetic acid solution at 0.032% (*w*/*v*) concentration. Sodium alginate was solubilised in water at 0.2% (*w*/*v*) concentration. Cu-product (0.6%, *w*/*w*) was dispersed in sodium alginate solution and stirred at 20°C. Chitosan solution was injected dropwise (syringe inner diameter of 0.40 mm) into a Cu-product/alginate dispersion and stirred for 1 h at 20°C. The weight ratio between the Cu-product/alginate dispersion and the chitosan solution was 1:1. The stable copper-loaded alginate/chitosan viscous dispersions were stored at 4°C until further studies.

Size determination

a) Hydrodynamic diameter and zeta potential measurements

The hydrodynamic diameter (d_h) and zeta-potential (ζ) were determined by dynamic light scattering (DLS) and laser Doppler anemometry (LDA), respectively (Zetasizer 3000 HSA, Malvern Instruments). The size measurements were performed at a scat-

tering angle of 90° and at a temperature of 25°C. The hydrodynamic diameter was calculated from the autocorrelation function of the intensity of light scattered by particles. For the zeta potential measurements, samples were placed in an electrophoretic cell, where a potential of 150mV was established. The results are presented as the mean of six determinations ± SD.

b) Light microscope

The size of microcapsules obtained was determined by using an light microscope (Olympus) equipped with i) a calibrated eye-piece (millimetre size capsules) or ii) with a Camedia digital camera C-4040, using quickPHOTO Camera 2.1. program (micrometer capsules). Diameters of about 300 capsules were measured.

In vitro release of copper

0.5 g of copper-loaded alginate/chitosan mixed capsules was dispersed in 10 ml of deionised water (pH 7) and left at room temperature without stirring for the duration of experiment. The release of copper from capsules produced blue colouring of the water medium and the absorbance was measured with an UV-VIS spectrophotometer (Shimadzu, UV-1700, PharmaSpec, $\lambda = 610$ nm). The results are presented as the mean of three determinations ± SD.

Result and discussion

Microcapsulation using only chitosan (preparation a) provides stable dispersion of copper-loaded microcapsules (7-11 µm, Figure 1) with positive zeta-potential (Table 1).

It is worth to notice that positive charge of chitosan microcapsules enables better bioadhesion on leaves which enables prolonged presence of copper on the leaf surface, as shown by Hadwiger et al. (2006). In addition, the prolonged release (as reported below) of copper from microcapsules and its prolonged presence on the leaves may lead to reduced levels of copper needed for sufficient crop protection (Hadwiger et al., 2006). On the other hand the usage of chitosan has one drawback and that is acetic environment (phytotoxicity) needed for its solubilisation. Neutralisation of chitosan solution is accompanied with precipitation of chitosan at around pH 6.5 (depending on molecular weight) and destruction of microcapsules. Therefore, alginate was introduced in order to prepare stable (upon neutralisation) microcapsules solution.

Table 1. Size and zeta-potential of prepared encapsulated copper formulations

Sample	Diameter	ζ / mV
Preparation a) Copper-loaded chitosan-based microcapsules (dispersed in 10 mM NaCl; weight ratio 1:10),	7 ± 11 µm⁺	+37.7 ± 1.1
Preparation b) Copper-loaded alginate/chitosan mixed capsules	1 ± 0,1 mm	-
Preparation c) Copper-loaded alginate/chitosan viscous microcapsules dispersions (diluted with 10 mM NaCl; weight ratio 1:10),	1,24 ± 0,01 µm*	-38.2 ± 0.3

*DLS measurement; ⁺Light microscope

Copper-loaded alginate/chitosan mixed capsules (preparation b) were generally spherical in nature and storage stable. Mixed capsules prepared are shown on Figure 2.

The average size of capsules were 1 mm ± 0.1 mm. Polyanionic alginate reacted during preparation with polycationic chitosan producing a complex polyelectrolyte system with incorporated copper. The *in vitro* release of a copper from such capsules (Figure 3) was determined, as described in experimental section, photometrically for a period of about six weeks.

The maximum release was achieved after that period of time and denoted as 100 percent. Smaller values were than calculated as percent of maximum release.

Figure 4 shows amount (in %) of copper released from capsules against time with the curve that does not fit the zero or the

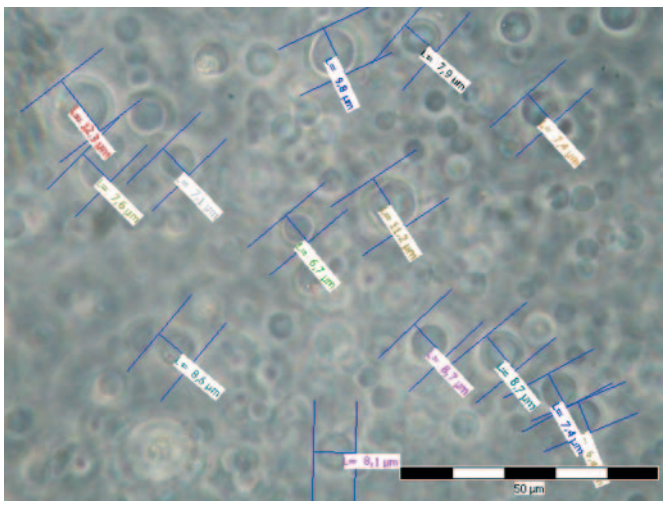

Figure 1. Size determination of copper-loaded chitosan based microcapsules

Figure 2. Mixed copper-loaded alginate/chitosan capsules

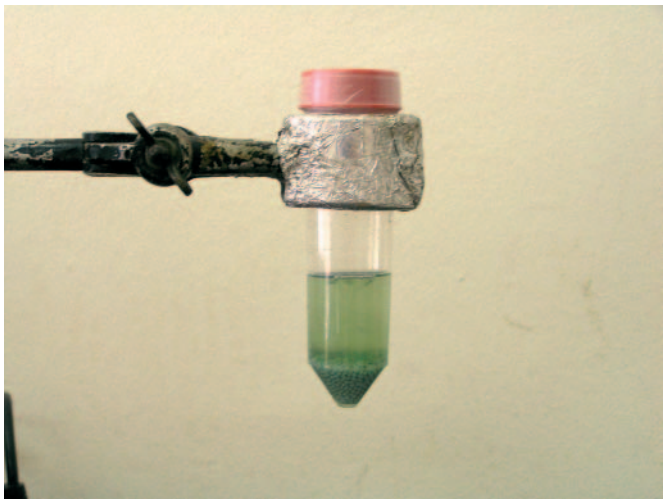

Figure 3. Colouring of deionisated water in contact with copper-loaded alginate/chitosan capsules

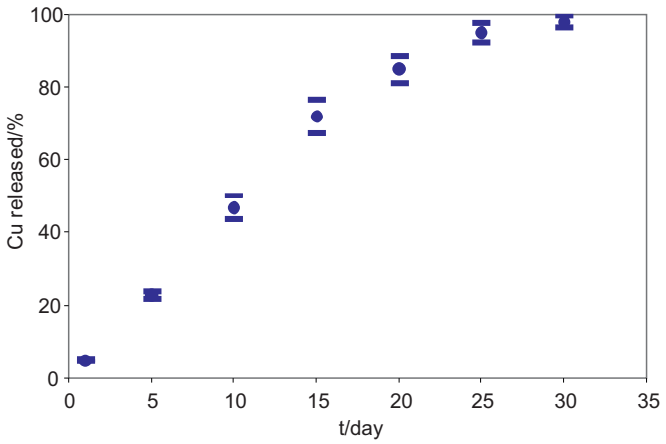

Figure 4. *In vitro* release of copper from copper-loaded alginate/chitosan capsules. Mean percent release ± SD

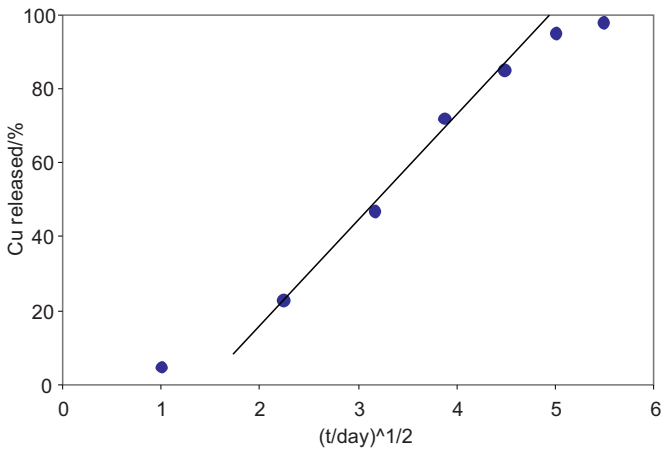

Figure 5. Higuchi diagram of copper release from alginate/chitosan capsules

first order release, implying that capsules can not be considered as a typical classical capsules consisting of a polymeric wall and core medium (Jalšenjak, 1992).

Figure 5 is a Higuchi diagram (percent release versus square root of time) producing a linear graph between 20 and about 80 percent of released substance. These results indicate that the release is from so called matrix (monolithic) entity where the encapsulated material and polymeric materials are mixed. The linear plot can be given with simplified equation were the amount released is proportional to the Higuchi equation (i.e. Higuchi constant of release rate x $t^{1/2}$). These findings are in accordance with polymeric nature of polyelectrolyte used.

The mechanical strength of mixed capsules during manipulation was satisfactory and no evident change was observed after several weeks of storage. When subjected to the freeze-drying process capsules shrinked without noticeable pores. The dried capsules can be resuspended in deionised water and used, for example, in vineyard. Application of such system, due to its millimetre size, can become somewhat unpractical, so by using different method of preparation (preparation c) the copper-loaded mixed alginate/chitosan microcapsules were prepared. Mean hydrodynamic diameter of microcapsules was about 1.2 μm (Table 1), allowing simple spraying of sample. Although, zeta-potential of such microcapsules was negative (Table 1) it should be noticed that zeta-potential of mixed alginate/chitosan microcapsules can be easily modified by changing alginate/chitosan mass ratio during preparation or with slight change of pH, where positive zeta potential of microcapsules is usually obtained at pH 7-8 (Saether et al., 2008).

Conclusion

We have presented three different preparations of copper-loaded mili- and microcapsules potentially useful in crop protection. Encapsulation of copper into a chitosan or chitosan/alginate complex polyelectrolyte system enables prolonged release of copper that is helpful whenever reduced level and/or prolonged presence of copper on the leaf surface is required. It seems that copper-loaded chitosan or chitosan/alginate microcapsules systems can be especially suitable in case of grapevine protection and that is worth to investigate in further research.

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