This manuscript was published as formal in:

Carbohydrate Polymers, 223:115040, 2019, 115040

DOI: 10.1016/j.carbpol.2019.115040

https://doi.org/10.1016/j.carbpol.2019.115040

***Title*:**

Designing chitosan based eco-friendly multifunctional soil conditioner systems with urea controlled release and water retention

Manuela Maria Iftime[1*], Gabriela Liliana Ailiesei[1], Elena Ungureanu[2], Luminita Marin[1*]

[1]Petru Poni Institute of Macromolecular Chemistry, Grigore Ghica Voda Alley, Iasi, Romania

[2] "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine, M. Sadoveanu Alley, Iasi, Romania

ciobanum@icmpp.ro; lmarin@icmpp.ro

Abstract

The paper reports new soil conditioner systems obtained by *in situ* hydrogelation of chitosan with salicylaldehyde in the presence of urea fertilizer, designed to address both fertilization and water retention of the soil. The new systems were structural, supramolecular and morphological characterized by FTIR spectroscopy, XRD diffraction, POM and SEM microscopy. The rate of urea release has been investigated by NMR analysis and the release mechanism has been assessed by fitting five mathematical models. The formulations showed high water absorbency of 68g/g, and they induced water holding capacity in soil up to 154% and an increment of the nitrogen content in soil to almost double, leading to a growth of plants with almost 70% higher compared to the reference soil. All these data revealed the new systems as new multifunctional soil conditioner ecoproducts capable to address both fertilizing and water retention issues, with high potential of application for sustainable agriculture.

Keywords: hydrogels, chitosan, salicylaldehyde, urea, Schiff base, controlled release, eco-design

1. Introduction

Soil fertilization is the most popular strategy to improve the agricultural productivity, imposed by the demographic changes (Hargreaves, Adl & Warman, 2008). Urea is one of the most used fertilizers due to its high nitrogen content and low cost (Azeem, KuShaari, Man, Basit & Thanh, 2014). However, due to its high volatility and high solubility in water, only a small percentage can be effectively absorbed by crops, most of it being lost by volatilization, immobilization, denitrification and leaching processes, leading to ecological and economic issues (Liu et al., 2013). A pathway for a more efficient exploitation of urea was identified in the development of systems for controlled release. An overview of the literature data evidenced different controlled release systems developed in the recent years: deposition on organic/inorganic functional materials, coating with polymers, encapsulation in matrices, copolymerization *via* immolable bonds (Naz & Sulaiman, 2016; Yang, An, Wang, Kan & Jin, 2017; Zhao et al., 2010). A large variety of synthetic polymers were used to build these systems, and gave good results in terms of urea prolonged release, but also showed high cost and lack of biodegradability, limiting their application. To overcome these drawbacks, biopolymer based formulations were designed and investigated as an eco-friendly alternative to those based on synthetic polymers. Polysaccharides are at the forefront of these researches due to their natural origin which confer them biocompatibility, biodegradability and harmless for leaving beings (Campos, Oliveira, Fraceto &

Singh, 2015; Corradini, Moura & Matoso, 2010; Guilherme et al., 2015; Majeed, Ramli, Mansor & Man, 2015; Ni, Liu & Lu, 2009; Wu & Liu, 2008). Moreover, they are originating from renewable resources, their use contributing to the preservation of the non-renewable resources and pollution prevention. Among them, chitosan is a prototypical polysaccharide which demonstrated antiviral and antifungal activity in plants and it induces abiotic and biotic stress tolerance in various horticulture crops (Iriti & Varoni, 2015; Malerba & Cerana, 2016). In addition, it is a nitrogen source for agricultural valorization, stimulating the plant growth (Pichyangkura & Chadchawanb, 2015).

Besides nutrients, the basic requirement for plant growth is the water retention in soil, especially for arid areas. Multifunctional formulations which are capable to release nutrients in a controlled manner and also to control the moisture of the soil are desirable for an improved agricultural production. Hydrogels are proper materials for this purpose, as they can encapsulate nutrients and adsorb a large amount of water, reducing water run-off and compaction rate and improving the soil permeability and infiltration rate (Abobatta, 2018; Kato et al., 2017). Used in many systems for delivery of bioactive compounds, the chitosan based hydrogels were less used as soil conditioners (Narayanan & Dhamodharan, 2015; Perez & Francois, 2016).

In the last years, our group developed a new strategy of chitosan hydrogelation with monoaldehydes, which allows the preparation of hydrogels with particular morphology and properties controlled by the nature of the aldehyde (Ailincai et al., 2016; Bejan, Ailincai, Simionescu & Marin, 2018; Craciun, Mititelu-Tartau, Pinteala & Marin, 2019; Iftime, Morariu & Marin, 2017; Iftime & Marin, 2018; Marin, Ailincai, Morariu & Tartau-Mititelu, 2017; Olaru et al., 2018). The use of the salicylaldehdye led to hydrogels with excellent mechanical properties, self-healing ability and outstanding swelling degree (Iftime et al., 2017). Recalling the good properties of chitosan and considering the valuable effects brought by hydrogels, we envisaged the possible profit of a design based on these hydrogels loaded with urea as multifunctional materials capable to fertilize and retain water in soil.

In line with these premises we designed and prepared soil conditioner systems by *in situ* hydrogelation of chitosan with salicylaldehyde in the presence of urea. The *in situ* hydrogelation was chosen as method of urea encapsulation, targeting its fine dispersion into hydrogels, with potential to slow down the release. The peculiarities of formation of the new formulations were investigated by FTIR spectroscopy, X-ray diffraction, SEM and POM microscopy. The urea release was investigated *in vitro* and the capability to retain water was measured by monitoring the water absorbency and largest water holding parameters. Preliminary evaluation of the

fertilizing ability on tomato seedlings indicated the new formulations as promising soil conditioner eco-products.

2. Materials and methods

2.1 Materials

Low molecular weight chitosan (314 kDa, DA=87%), salicylaldehyde (98%), urea (98%), ethanol (99.8%), and glacial acetic acid (99.8%) from Aldrich were used as received.

2.2 Preparation of the soil conditioner formulations

A series of formulations with different crosslinking degrees (NH_2/CHO ratio of glucosamine units of chitosan and salicylaldehyde from 1/1 up to 3/1) and different content of fertilizer (from 0 to 66%) were prepared by encapsulation of urea into salicyl-imine-chitosan hydrogels by *in situ* hydrogelation (Scheme 1), as follows. (1) 0.1 g chitosan (5.05×10^{-4} mmol glucosamine units) was dissolved into a mixture of 4.9 mL water and 35 µL acetic acid to give a 2.02% solution, which was then heated at 50 ^{o}C. (2) Different amounts of salicylaldehyde and urea (Table 1) were dissolved into a mixture of 100 µL water with ethanol to give a 1% solution, which was (3) slowly dropped into the chitosan one, under vigorous stirring at 50 ^{o}C. The codes of the formulations with different molar ratios of the NH_2/CHO functional groups and different urea content were given in Table 1. The visual formation of hydrogels was observed after 2-3 minutes for the **CS1-Ux** and **CS1.5-Ux** formulations; 8-10 minutes for **CS2-Ux**; and 2 days for **CS3-Ux**. Reference salicyl-imine-chitosan hydrogels without urea were prepared too, when hydrogelation was observed after 3-4 minutes for **CS1-U0** and **CS1.5-U0**; 2 hours for **CS2-U0**, while the **CS3-U0** transformed into a viscous liquid which still flew after two weeks. The formulations appeared as transparent yellowish semisolid materials with smooth texture, similar to the reference hydrogels (Scheme 1). Next, they were kept uncovered over 7-9 days up to the initial volume of chitosan solution was reached and after that, they were subjected to lyophilisation in order to obtain the corresponding xerogels.

Table 1. Composition of the formulations and their codes

Code	**CS1-U0**	**CS1-U0.5**	**CS1-U1**	**CS1-U2**
NH_2/CHO ratio (CSx)	**1:1 (CS1)**			
Urea content % (Ux)	**0 (U0)**	**33 (U0.5)**	**50 (U1)**	**66 (U2)**
Chitosan (mg)	100			
Glucosamine (mmol)	5.05051×10^{-4}			
SA (mg/mmol)	$62 / 5.05051 \times 10^{-4}$			
Urea (mg)	0	82.1	162	324
Bidistilled water (mL)	5			
Acetic acid (µL)	35			
Ethanol (mL)	6.2			
Xerogel weight (mg)	161	238	322	484
Code	**CS1.5-U0**	**CS1.5-U0.5**	**CS1.5-U1**	**CS1.5-U2**
NH_2/CHO ratio (CSx)	**1.5:1 (CS1.5)**			
Urea content % (Ux)	**0 (U0)**	**33 (U0.5)**	**50 (U1)**	**66 (U2)**
Chitosan (mg)	100			
Glucosamine (mmol)	5.05×10^{-4}			
SA (mg/mmol)	$41 / 3.36 \times 10^{-4}$			
Urea (mg)	0	70.5	141	282
Ethanol (mL)	4.1			
Bidistilled water (mL)	5			
Acetic acid (µL)	35			
Xerogel weight (mg)	140	210	280	421
Code	**CS2-U0**	**CS2-U0.5**	**CS2-U1**	**CS2-U2**
NH_2/CHO ratio (CSx)	**2:1 (CS2)**			
Urea content % (Ux)	**0 (U0)**	**33 (U0.5)**	**50 (U1)**	**66 (U2)**
Chitosan (mg)	100			
Glucosamine (mmol)	5.05×10^{-4}			
SA (mg/mmol)	$31 / 2.52 \times 10^{-4}$			
Urea (mg)	0	65	131	262
Ethanol (mL)	3.1			
Bidistilled water (mL)	5			
Acetic acid (µL)	35			
Xerogel weight (mg)	130	195	261	392
Code	**CS3-U0**	**CS3-U0.5**	**CS3-U1**	**CS3-U2**
NH_2/CHO ratio (CSx)	**3:1 (CS3)**			
Urea content % (Ux)	**0 (U0)**	**33 (U0.5)**	**50 (U1)**	**66 (U2)**
Chitosan (mg)	100			
Glucosamine (mmol)	5.05×10^{-4}			
SA (mg/mmol)	$021 / 1.68 \times 10^{-4}$			
Urea (mg)	0	60.5	121	242
Ethanol (mL)	2.1			
Bidistilled water (mL)	5			
Acetic acid (µL)	35			
Xerogel weight (mg)	120	180	241	359

The xerogels weight was almost similar with that of the initial reagents, indicating no mass loss during lyophilisation (Table 1). The sample prepared with the largest amount of salicylaldehyde (**CS1-Ux**) was brittle and had a heterogeneous appearance, while those with a lower amount (**CS1.5-Ux**, **CS2-Ux** and **CS3-Ux**) were homogeneous with a porous aspect.

2.3 Methods and equipment

The formulations and reference hydrogels were lyophilized using a Labconco FreeZone Freeze Dry System equipment, for 24 h at −54 ºC and 1.512 mbar, after the prior freezing in liquid nitrogen.

Fourier-transform infrared (FTIR) spectra were recorded with a FT-IR Bruker Vertex 70 Spectrophotometer, by ATR technique, and processed using OPUS 6.5 software (see Supporting Information, Figure S1).

Wide angle X-ray diffraction (WXRD) was performed on a Bruker D8 Avance diffractometer with Ni-filtered Cu-Ka radiation (λ = 0.1541 nm), in the range of 2−40 º (2 theta). The dimension of the imine clusters (D) was calculated applying the Debye–Scherrer formula for the reflection peak around 6 º: $D = K\lambda/\beta\cos\theta$, where D is the average diameter in nm, k is the shape factor (k¼=0.9); λ is the X-ray wavelength; β is the full width at half maximum of the diffraction in radians, and θ is Bragg's diffraction angle (Samoila et al., 2015). Debye–Scherrer equation was also applied to the reflections around 22 and 29 º, respectively, in order to calculate the dimension of the urea crystals.

The morphology of the formulations was investigated with a field emission ***Scanning Electron Microscope*** (SEM) EDAX – Quanta 200 at accelerated electron energy of 20 KeV.

In vitro release behavior of urea from the formulations was investigated on xerogel samples containing 50 mg of urea, at room temperature, in distilled water, as follows: the samples were immersed into vials containing 10 mL of distilled water. At certain times, 1 mL of supernatant was withdrawn from the vials and replaced with 1 mL of distilled water. This procedure was applied during 35 days. The quantity of released urea was determined by ^{1}H-NMR spectroscopy, by fitting on a calibration curve previously generated for urea, as detailed into supporting information Figure S2). The cumulative urea release was calculated with eq.: $urea\% = [(10Cn+2\Sigma Cn-1)/m_o]x100$, where Cn and Cn-1 represent the concentrations of the urea in supernatant after n and n-1 withdrawing steps, respectively, and m_o=50 mg, corresponding to the urea in the initial samples.

In order to evaluate ***the mechanism of the urea release***, the data were fitted on the equations of the Korsmeyer-Peppas, Zero order, First order, Higuchi and Hixson-Crowell mathematical models (Craciun et al., 2019, Lin & Metters, 2006).

The ***water absorbency*** (**WA**) of the formulations was determined by gravimetric method, as follows. Samples containing the same amount of hydrogel matrix ($30x10^{-3}$g **CS2-U0.5**; $40x10^{-3}$g **CS2-U1** and $60x10^{-3}$g **CS2-U2**) were immersed into 10 mL distilled water and allowed to soak at room temperature for 90 min. The swollen samples were weighed after removing the water on the surface with a tissue paper. The water absorbency of the formulations was calculated applying the equation: $WA= W_s-W_d/W_d$, where W_d and W_s are the weight of the samples in dried and swollen state, respectively (Ni, Liu & Lü, 2009).

The ***hydrolytic stability*** of the hydrogel matrix was investigated by immersing pieces of xerogels of 10 mg in buffer solutions of different pH, from 2.5 up to 10, over 15 days. The moment of their complete dissolution was noted. After 15 days, the hydrogels were taken off, lyophilized, then weighed and the loss percent was calculated.

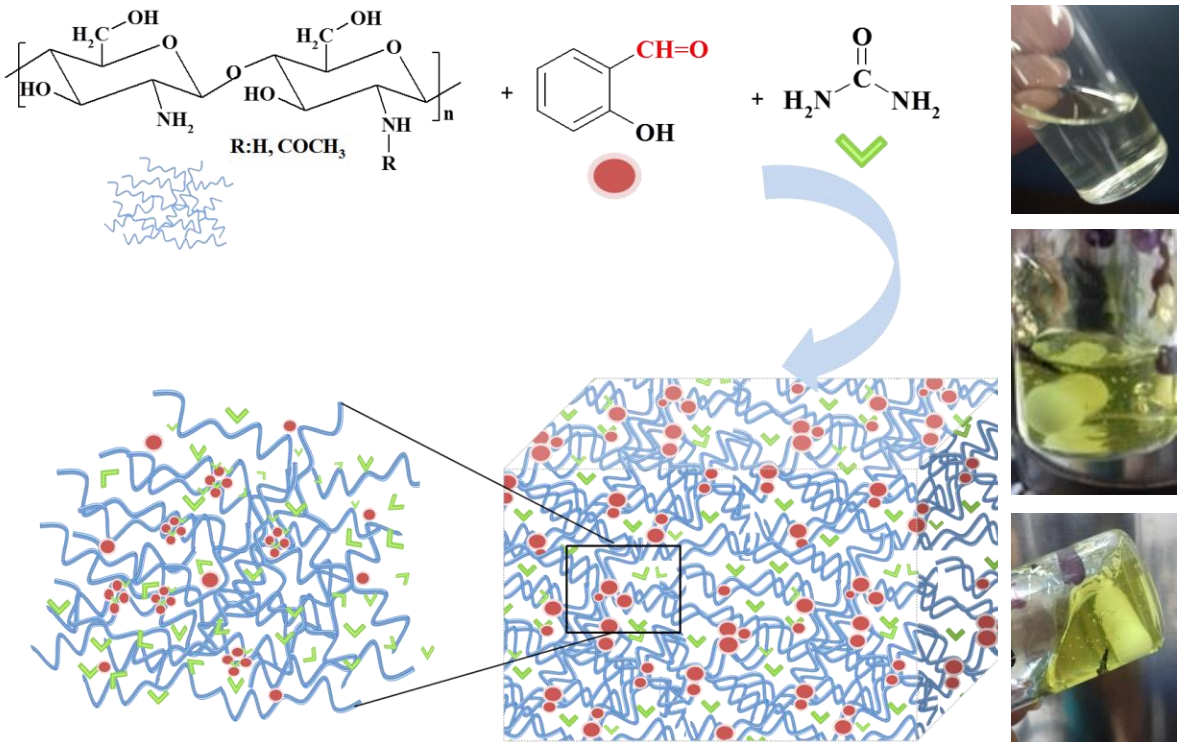
For practical applications, the ***largest water-holding ratio***, ***dynamic of the nitrogen***, and ***growth indexes*** of tomato seeds were calculated in laboratory experiments on *black peat* soil. The detailed experimental procedure was described in Supporting Information.

Statistical analysis

The determinations were done in triplicate for all the measurements, and the average value calculated (mean±SD; n=3) was taken as result.

3. Results and discussion

Four series of soil conditioner formulations were prepared by *in situ* hydrogelation of chitosan with salicylaldehyde in the presence of urea. It was expected that hydrogelation to take place due to the ordering of the newly formed imine units into supramolecular clusters, which play the role of crosslinking nodes, as already proved in our previous paper (Scheme 1) (Iftime et al., 2017). The four series of formulations were obtained by varying the molar ratio of the functional groups of chitosan and salicyladehyde, NH_2/CHO = 3/1; 2/1; 1.5/1; 1/1, with the aim to achieve different crosslinking degrees. Each series consists in four formulations, for which the content of urea was varied from 0 up to 66 %. Thus, 16 formulations were resulted, different among them by the crosslinking degree and the content of fertilizer (Table 1).



Scheme 1. Synthesis of the urea soil conditioners formulations

A possible reaction of urea with chitosan *via* imine formation during the hydrogelation has been excluded by ^{1}H-NMR spectroscopy on a model reaction system (Supporting Information-Figure S3).

The structural and supramolecular peculiarities of the formulations were investigated by FTIR spectroscopy, wide angle X-ray diffraction (WXRD) and polarized optical light microscopy (POM).

3.1 Structural investigation of the formulations by FTIR

The FTIR spectra of the formulations were recorded in order to investigate the hydrogelation mechanism of the systems and to have an insight on the pathway of urea encapsulation into them. They were assessed in comparison with the reference salicyl-imine-chitosan hydrogels and urea (Figure 1; Supporting Information-Figure S4). The hydrogelation of chitosan with salicylaldehyde was demonstrated by FTIR spectra by (i) the presence of the characteristic band of imine linkage as an intense, sharp band at 1628-1630 cm^{-1} and (ii) the modifications of the broad band from 3000 to 3700 cm^{-1}, attributed to the overlapped stretching bands of the O-H and N-H bonds and the intra- and inter-molecular H-bonds. These spectral peculiarities were in line with the formation of supramolecular clusters of imine units which played the role of chitosan crosslinkers (Iftime et al., 2017). The FTIR spectra of the formulations showed the bands

characteristic to the reference hydrogels (**CSx-U0**) and urea, with slight modifications in position and intensity, indicating the preservation of the hydrogelation mechanism and the occurring of physical interactions between the two components.

The stretching bands of the C=O and N-H bonds into urea (1670 cm^{-1} and 1146 cm^{-1}, respectively) appeared shifted to higher wavenumbers (around 6 cm^{-1}) into the spectra of the formulations, pointing for their involvement into physical bonds with the hydrogel, extremely likely H-bonds as also evidenced for other chitosan – urea systems (Araújo, Romao, Doumer & Mangrich, 2017). No obvious diminishing of intensity of the C=O band was noted, suggesting the absence of condensation reaction with amine groups of chitosan, as the model reaction indicated too. As in the fingerprint domain appeared overlapped bands from hydrogel and urea, the subtraction of the urea spectrum from that of the formulations was applied, when the imine band was clearly evidenced at 1629 cm^{-1}, similar to the reference hydrogels (Supporting Information-Figure S1). The 3000–3500 cm^{-1} spectral domain of the formulations became dominated by the vibration bands of amine groups of urea, while the bands characteristic to hydroxyl and methylene units drastically decreased in intensity, in agreement with the dominant content of urea in the detriment of chitosan.

All these FTIR data indicated the anchoring of the urea into the formulations by physical interactions, during the hydrogelation. Correlating FTIR data with the experimental observations, it appears that the occurring of the physical bonds between urea and chitosan favored the hydrogelation processes.

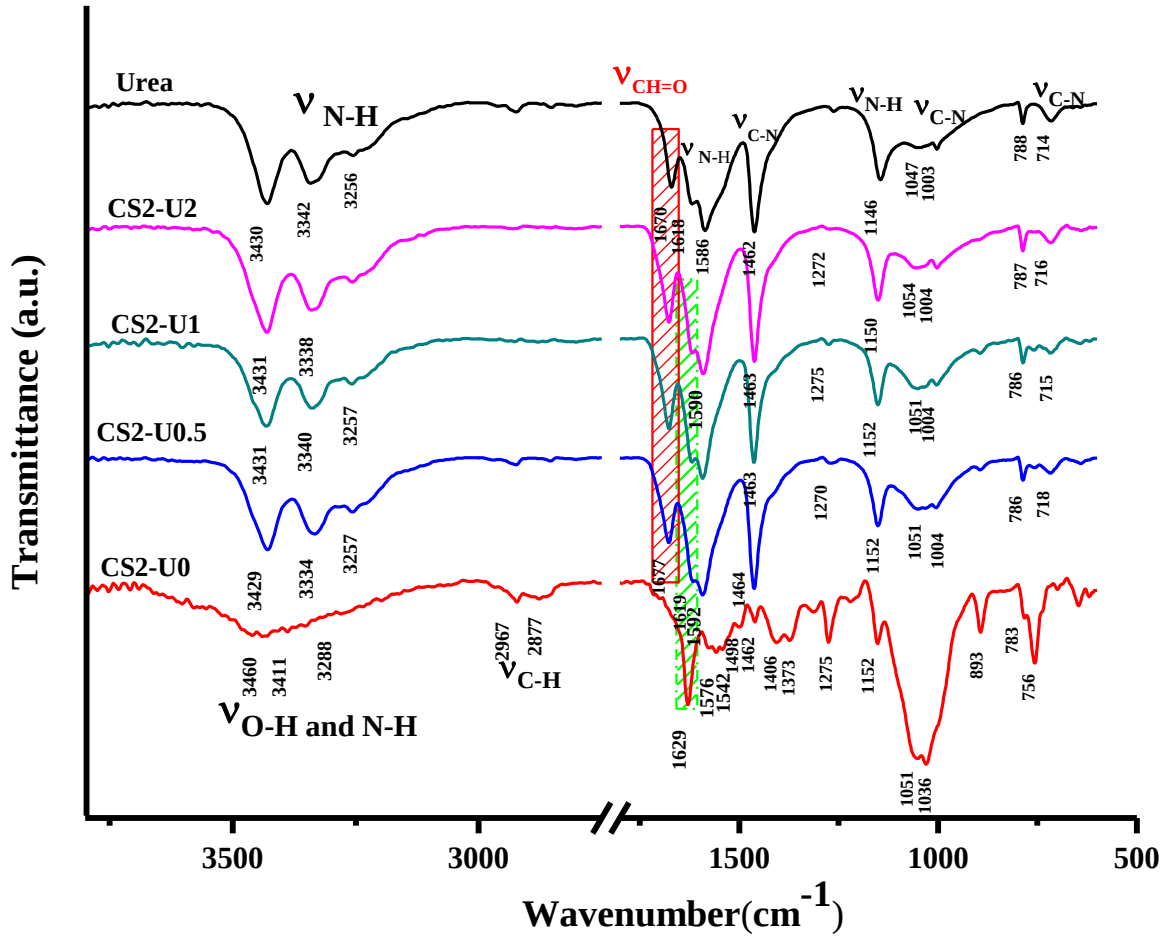


Fig. 1. FTIR spectra of a series of formulations (**CS2-Ux**), the reference xerogel (**CS2-U0**), and urea

3.2 Supramolecular analysis of the formulations by wide angle X-ray diffraction (WXRD)

The formation of the salicyl-imine based crosslinking nodes into the reference hydrogels has been proved by WXRD mainly by the appearance of the reflection at a lower angle (6.22–6.6^{o}), characteristic to the inter-layer distance of their layered supramolecular architecture (Iftime et al., 2017). The supramolecular architecture of the crosslinking nodes was further confirmed by the presence of broad reflections at wide angle (14 and 20^{o}, respectively) corresponding to the inter-molecular and inter-chain distances inside them.

The formulations showed the distinct reflection peak at lower angles (6.12–6.7^{o}), with slight variations of its maximum compared to the reference hydrogels, reflecting the environment influence, e.g. intermolecular forces between the urea and chitosan, the presence of urea crystals (Figure 2; Supporting Information-Figure S5). The structural parameters were given in Supporting Information-Table S1. To have a quantitative insight on the formation of the crosslinking nodes, their size was calculated applying Scherer equation to the reflection band

characteristic to the inter-layer distance of the ordered clusters (6.12–6.7^{o}) (Samoila et al., 2015). The obtained results were given in Supporting Information-Table S2. For the reference hydrogels it was observed a variation of the diameter of the crosslinking nodes from 2.8 (**CS1-U0**, **CS3-U0**) to 3.5 (**CS1.5-U0**) and further to 4.2 nm (**CS2-U0**), in accordance with the balance between the density of imine units and the viscosity of the reaction system (see Supporting Information for more details). Interesting enough, except the **CS2-Ux** series, the presence of urea led to a slight increment of the size of crosslinking nodes, more pronounced in the case of the **CS1.5-Ux** series, suggesting that the physical forces developed between urea and chitosan facilitated the self-ordering of the imine units (Santos, Bacalhau, Pereira, Souza & Faez, 2015). It can be estimated that these forces led to a stiffening of the chitosan chains and thus a decreasing of its mobility, which favored the self-assembling of the newly formed imine units, and thus a faster hydrogelation.

Besides the reflection bands characteristic to the reference hydrogels, the WXRD diffractograms of the formulations show the reflection bands characteristic to the urea (Figure 2; Supporting Information-Figure S5). Comparing the crystallographic profile of pure urea, attributed to a tetragonal system (Madhurambal, Mariappan & Mojumdar, 2010) to that of the studied formulations, slight shifting of the reflection bands and changes in their intensity were noted (Figure 2; Supporting Information-Table S1). Remarkable it was the shifting of the reflection band from 40.52° (d=2.37 Å) to 41.58° (d=2.32 Å), and the increase in intensity of the reflections from 24.58, 29.38 and 37.01°. Considering that urea is a hydrogen bonded molecular crystal for which the reflections correspond to the different lengths of the in-plane and out-of-plane hydrogen bonds (Gatti, Saunders & Roetti, 1994), these changes can be speculated to occur under the influence of the chitosan–urea physical forces leading to rich-defect urea crystals (Craciun et al., 2019).

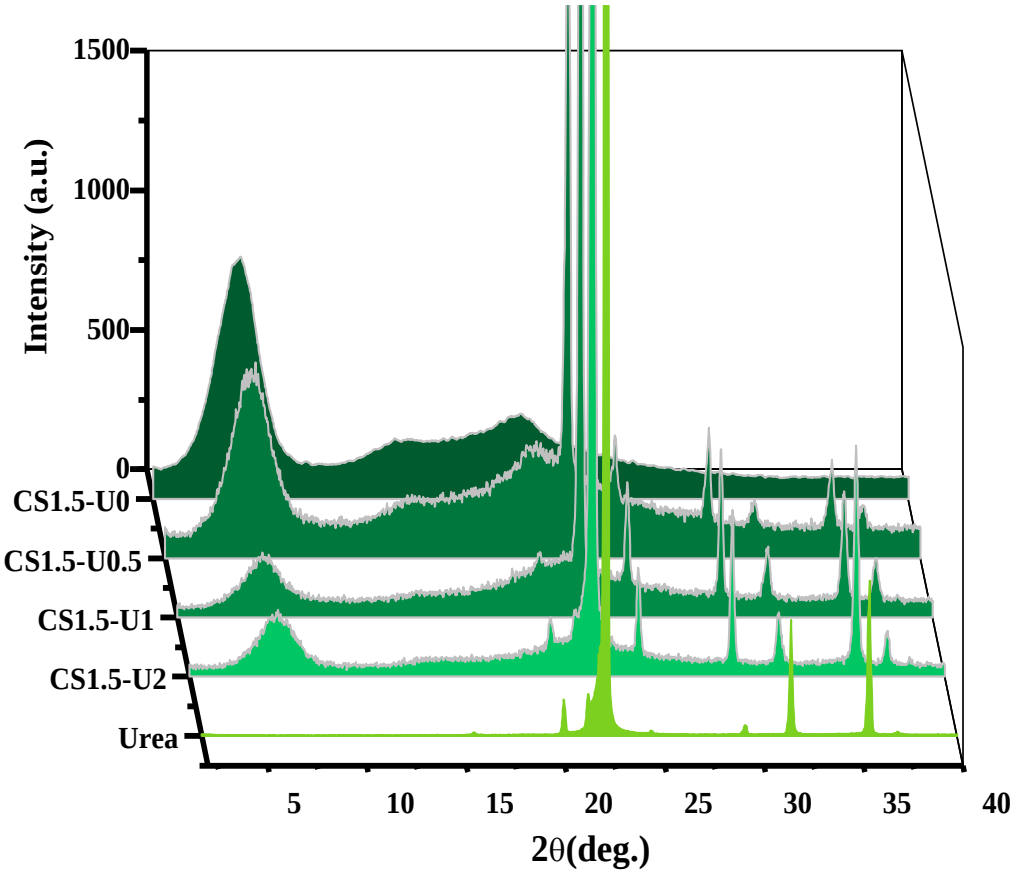


Fig. 2. X-ray diffractograms of the formulations **CS1.5-Ux** and urea

Applying Scherrer equation to the reflections around 22.4 and 29.3 º, the size of the urea crystals into formulations was calculated, giving a diameter of urea crystals ranging from 28 to 39 nm (Supporting Information-Table S2). The dynamic of their size followed a similar trend to that of the imine clusters; smaller crystals were obtained for the highest and lowest crosslinking degree and bigger ones for the intermediates values, indicating similar driving forces of ordering. For each series, the size of the urea crystals increased with its content.

3.3 Formulation morphology

The formulation morphology was investigated by SEM, which also provided an insight on the urea incorporation (Figure 3). A correlation between morphology and crosslinking degree (reflected by the content of salicylaldehyde) and the content of urea was evident.

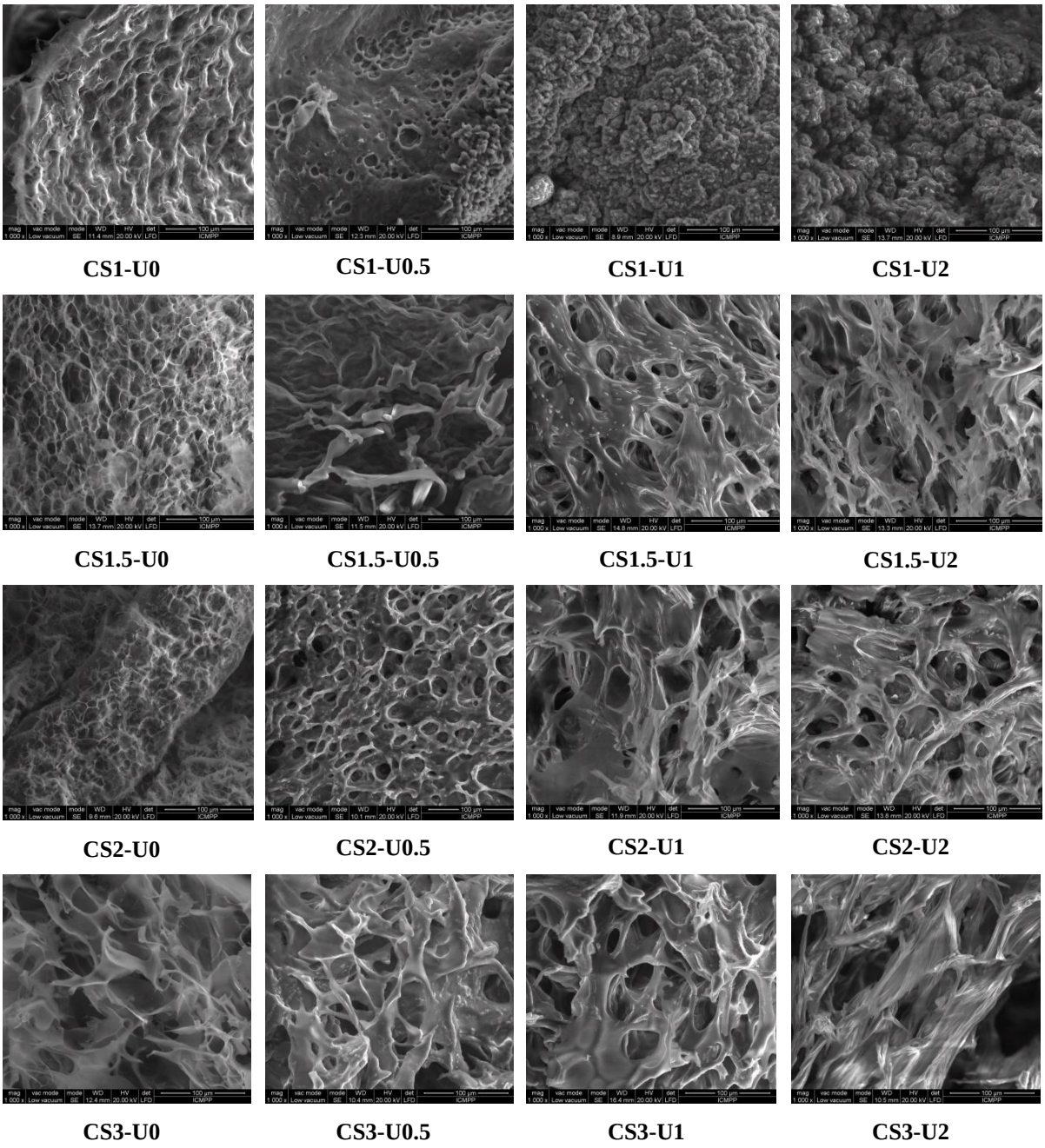


Fig. 3. Representative SEM images of the formulations and reference hydrogels (**CS1-Ux**-**CS3-Ux**)

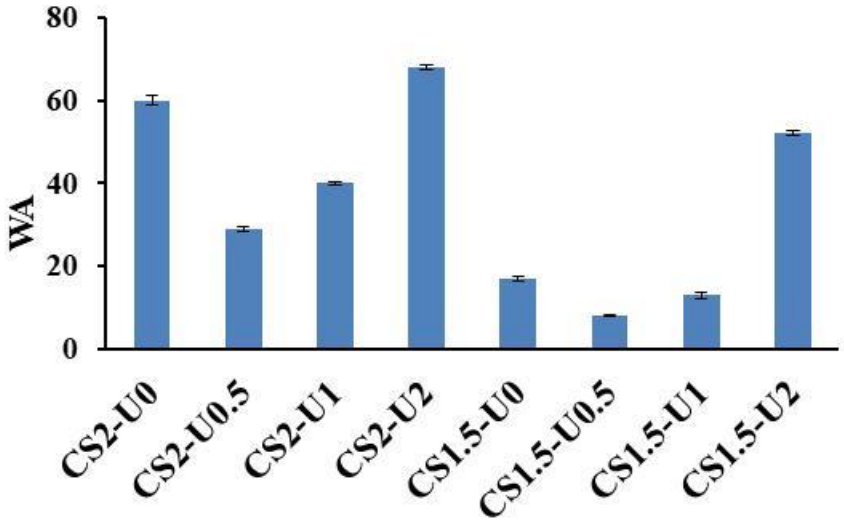
Generally speaking, the increasing of the crosslinking degree gave a denser structure while the increasing of the content of urea led to thicker pore walls. Thus, in the case of the **CS1-Ux** series, the increasing of the urea content led to an almost compact structure with small pores; the two series with a medium crosslinking degree (**CS1.5-Ux**, **CS2-Ux**) showed a homogeneous, porous morphology, with interconnected pores and rare visible urea crystals embedded into the pore walls; the series with the lower crosslinking degree (**CS3-Ux**) showed also a porous morphology, with obvious larger urea crystals, as its content increased.

Polarized light microscopy images further supported X-ray and SEM data, confirming the presence of urea crystals for the samples with the higher and the lower crosslinking degree (Supporting Information- Figure S6 d,f) and their absence for those with medium crosslinking density (Supporting Information- Figure S6 e), when a fine birefringent texture was visualized (Marin, Popescu, Zabulica, Uji-I & Fron, 2013).

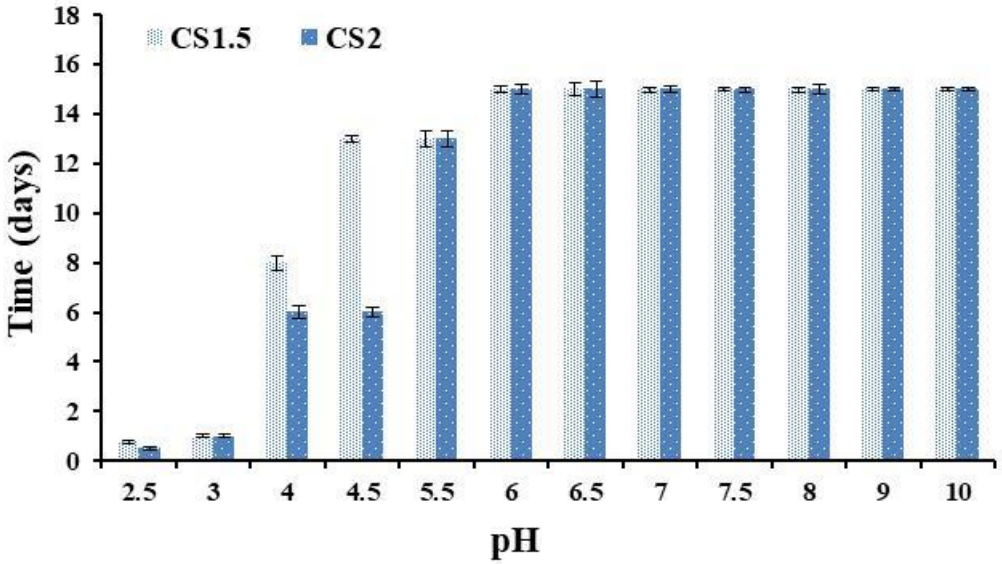
3.4 Water absorbency and hydrolytic stability of the formulations

The potential of the formulations to absorb water was investigated in comparison with the reference hydrogels. The formulations quickly swollen when immersed in distilled water and their hydrolytic stability and water absorbency (WA) correlated with the crosslinking density and urea content; a higher crosslinking density led to a higher hydrolytic stability but a lower absorption capacity. Thus, **CS1-Ux** samples were hydrolytic stable but presented the lowest absorption capacity, in agreement with their low porosity. Opposite, the **CS3-Ux** formulations swelled very fast, but they disintegrated simultaneously. The formulations with a medium crosslinking degree (**CS2-Ux** and **CS1.5-Ux**) proved the best balance of water absorbency/hydrolytic stability, with a **WA** from 68 to 9, while being hydrolytic stable on the entire period of investigation (35 days). **WA** increased as the content of urea increased, probably due to the increased number of pores resulted as the urea dissolved. In the case of **CS2-Ux** samples, **WA** increased progressively from 29 (**CS2-U0.5**) to 68 (**CS2-U2**), surpassing that of the reference hydrogel (Figure 4a).

As the hydrogel matrix is based on the imine linkage known as being pH responsive [Godoy-Alcántar, Yatsimirsky & Lehn, 2005; Tao, Liu, Zhang, Chi & Xu, 2018], which should influence the urea release kinetics, the hydrolytic stability was further investigated in media of different pH, ranging from 2.5 to 10. As can be seen in figure 4b the stability over time increased as the pH increased, the hydrogels immersed in media of pH higher than 6 still keeping their integrity after 15 days. The weighing after 15 days revealed mass loss from 14 to 56%, the hydrogels showing the best stability in media of neutral pH, superficial erosions occurring during the time (Supporting Information-Table S3).



a)



b)

Fig. 4. a) Water absorbency of the **CS2-Ux** and **CS1.5-Ux** formulations, in distilled water; b) Time stability of **CS2** and **CS1.5** hydrogel matrix in media of different pH during 15 days

3.5 *In vitro* release behavior of urea fertilizer. Release kinetics

The formulations with the best balance of water absorbency/hydrolytic stability, **CS2-Ux** and **CS1.5-Ux** series, were further investigated for their ability to act as matrix for controlled release of urea fertilizer, by measuring the *in vitro* release profile in distilled water, at room temperature during 35 days. The results were gathered in Figure 5a-e. All formulations released the encapsulated urea in three stages: (i) a burst effect in the first 5 hours, when up to 46% urea passed

in the water medium (Figure 5d); (ii) a slower release in the next 11 days, reaching 75% released urea (Figure 5c); and (iii) a slower continuous release in the next 23 days when almost all the urea passed in the water medium (Figure 5a, b; Supporting Information-Table S4).

Comparing the release rates of the six analyzed formulations, the influence of the crosslinking degree and urea content (which were reflected on the morphology and water absorbency) could be observed (Figure 5e). As expected, the highest release rate has been recorded for the samples with the highest content of urea (**CS2-U2**, **CS1.5-U2**), according to its encapsulation as larger crystals, less anchored into the matrix and thus subjected to a faster dissolution. **CS1.5-Ux** samples showed a slightly faster release compared to **CS2-Ux**, probably due to the fact that the more viscous medium during hydrogelation influenced the growth of defect-rich urea crystals, which were more susceptible to dissolution (Craciun et al., 2019). The highest amount of released urea has been noted for **CS2-Ux**, in agreement with their lower crosslinking degree which favored a higher swelling and thus easier urea dissolution and diffusion to the release medium.

The formulations didn't show visible changes during the investigation period of 35 days, and their FTIR spectra were similar to those of the reference hydrogels (Supporting Information-Figure S7), pointing for their stability and their further utility as soil conditioners, even after urea release.

To go in deeper details of the release mechanism of urea from the studied formulations, a mathematical analysis of the *in vitro* release profile has been performed. To proper asses the mechanism of urea release in our formulations, five different mathematical models were fitted on the *in vitro* release profile, on each of the three stages (Supporting Information-Table S5, S6, S7).

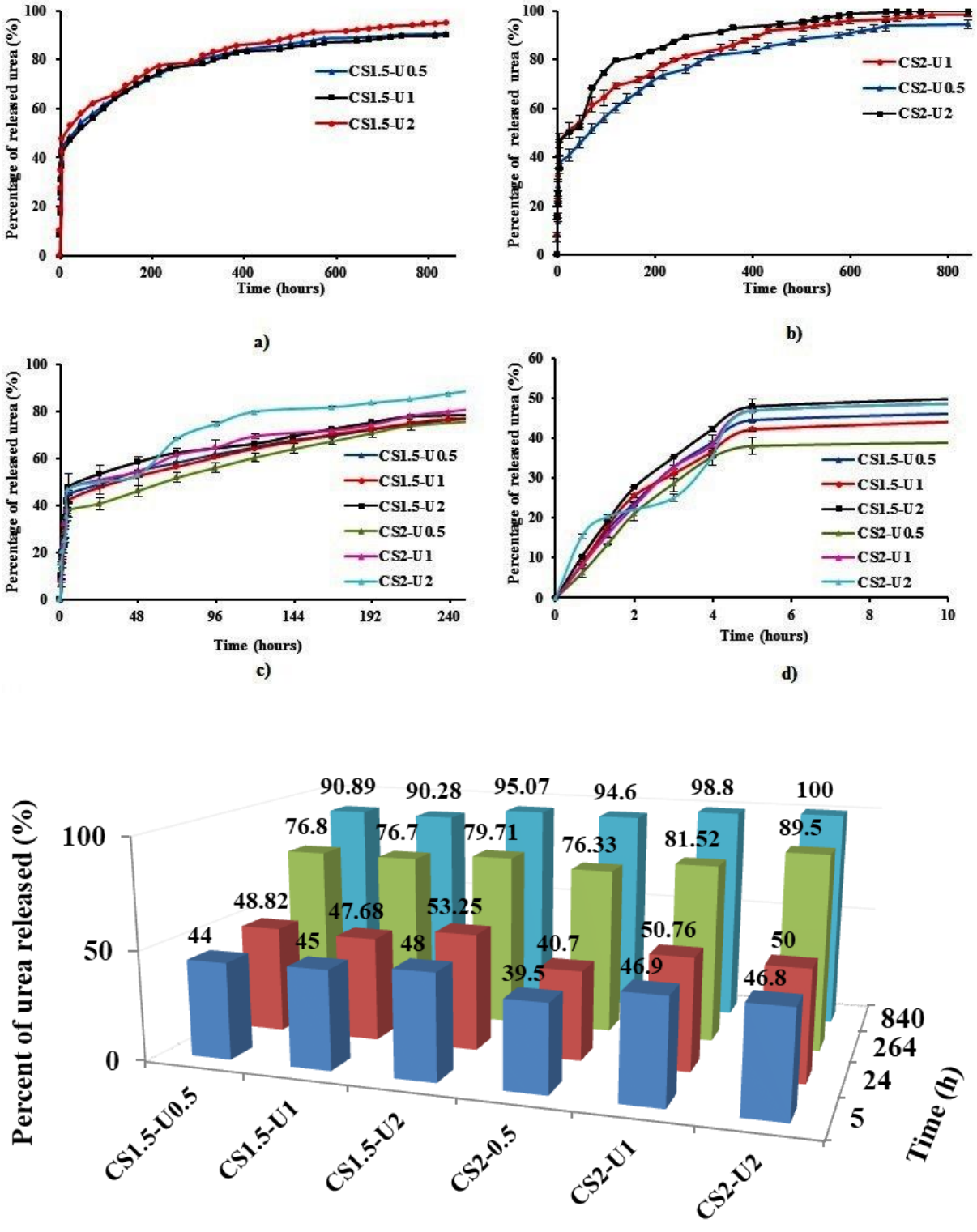


Fig. 5. Percentages of urea released during 35 days from the formulations (a,b,e) detailed for the first 10 days (c) and first 10 hours (d)

The graphical representation of the equations of the five mathematical models on the data obtained from the first stage of the *in vitro* release gave a high correlation coefficient (R^2=0.98–0.99) in the case of Zero Order, Higuchi and Korsmeyer-Peppas and lower correlation coefficient in the case of First order and Hixson-Crowell models, for all samples (Supporting Information-Table S5). This means that the mechanism of the first release stage can be described by these three models, as follows.

The excellent fitting of ***Zero Order*** model (R^2=0.98–0.99) indicates that urea release was influenced by the swelling transition of the hydrogel matrix in the first 5 hours of experiment. Differences in the proportionality constant (k_o) reflect the effect of the matrix on the speed of urea dissolution, which can be correlated with the strength of its anchoring into the matrix.

The good fitting of the **Higuchi** model (R^2=0.996–0.999) shows that urea release was controlled by the diffusion process. At this stage, the concentration of urea into the release medium (3 mg/mL) was much under the saturation concentration (1.04 g/mL), indicating that the encapsulation of the urea into the hydrogel matrix prevented its fast dissolution and promoted its slow diffusion towards the release medium. This can be attributed to the physical forces between the urea and hydrogel matrix which on the one hand anchored the urea crystals slowing down their dissolution and on the other hand bonded the molecules of urea slowing down their sink on inner-outer direction imposed by the concentration gradient.

Further, the fitting of the Korsmeyer-Peppas model (R^2=0.981–0.997) confirmed the influence of the hydrogel matrix, as the two previous models suggested, and gave information related to the type of diffusion. Except **CS2-U0.5** which gave an exponent n value of 0.98 indicating a case II transport, the other samples showed the n exponent values from 0.65 to 0.89, characteristic for a non-Fickian anomalous transport when the diffusion through the matrix was occurring simultaneously with the matrix swelling. This behavior was also revealed by other polysaccharides based nutrient carriers, which showed swelling in the first stage when the water molecules penetrate the hydrogel network and dissolve the nutrient, simultaneously (Guilherme et al., 2015). In our particular formulations, it is expected that urea movements into the hydrogel walls would exercise stress on them, leading to some degree of morphological changes, e.g. cracks, and subsequently to a change of the diffusivity.

The lack of fitting of the First Order Kinetic model (R^2=0.88–0.91) indicates no control of the amount of encapsulated urea onto its release, while the absence of fitting of the Hixon-Crowell model (R^2=0.92–0.95) shows that urea release is mainly controlled by its diffusion through the matrix and less by its dissolution velocity. Both features can be attributed to the presence of

larger crystals of urea less anchored into the matrix, susceptible to a faster dissolution in the first release stage.

In the second stage of the *in vitro* release, all the five mathematical models fitted very well on the obtained data (Supporting Information-Table S6) indicating some changes in the release mechanism. The good fitting of the First Order Kinetic model indicates that in this stage the amount of the urea remained into matrix plays an important role on its release rate. The fitting of the Hixon-Crowell model shows that the urea dissolution velocity surpasses the importance of its diffusion through the hydrogel matrix. Even if some studies recommend the use of the Korsmeyer Peppas model only for domains of up to a 60% of active compound released, it fitted very well on the second stage of urea release (60–75% released urea) and revealed a drastic decrease of the n to the range 0.14–0.33, indicating the turn of the urea diffusion to a Fickian pattern, in agreement with urea diffusion on the direction controlled by the concentration gradient. Worthy of consideration is also the drastic diminishing of the proportionality constants to close values for the second release stage, indicating an almost similar influence of the hydrogel matrix on the urea release (Supporting Information-Table S6). All these suggest that in the second stage, due to the morphological changes (e.g. swollen matrix, cracks into the pore walls), the diffusion of the urea molecules was facilitated, and thus the dissolution velocity became the driving force for urea sink on inner-to-outer direction. This can be related to the strong anchoring of the smaller crystals or even molecules of the remaining urea into matrix, slowing down its dissolution.

In the third second stage, except Korsmeyer Peppas, the fitting of all mathematical models failed (R^2=0.81–0.96) for almost all the samples (Supporting Information-Table S7), indicating that heterogeneous erosions of the matrix occurred and no control of the principal processes were longer available.

Summarizing, the urea release mechanism from salicyl-imine-chitosan hydrogels could be illustrated as follows. The soaking of the formulation samples in water allows them to swell and then transform into hydrogel. The water transferred into the crosslinked hydrogel, first through the pores and then penetrated the pore walls, dissolving the urea. In the first stage, larger urea crystals less anchored into the hydrogels matrix were susceptible to dissolution, followed in the second and third stage by the smaller urea crystals, better anchored. The dissolved urea diffused out from the matrix and then released through the dynamic exchange with free water. Considering the mechanism of urea release, it can be estimated that the soil moisture will guide its delivery in practical applications, as also demonstrated by other authors [Agehara, & Warncke, 2005].

Overall, the *in vitro* release profile of the studied formulations suggests that the salicyl-imine-chitosan hydrogels are appropriate matrixes for a sustained delivery of the urea fertilizer: an initial burst effect should help the growing of plants by a greater fertility dosage, followed by a constant release during the plant growth (Azeem et al., 2014).

Table 2 summarizes the values of water absorbency and percentage of urea released from other chitosan based formulations reported by other research groups. As can be seen, a sustained release of urea has been also achieved for other chitosan based hydrogels or microspheres, but water absorbency had significant lower values compared to the salicylimine-chitosan based formulations studied in this paper. Hence, the results obtained in this work are very encouraging and open up the possibility of using such materials as multifunctional soil conditioners at larger scale.

Table 2. Water absorbency (WA) and percentage of urea released from chitosan based materials

Formulation	**Performances**	**Reference**
Urea *in situ* encapsulated into salicylimine-chitosan hydrogels	WA = 68 g/g Burst effect ≅ 45% in first 5 h Prolonged release ≅100% after 35 days	the present study
Urea absorbed into chitosan based hydrogels (oxidized chitosan or itaconic acid grafted on chitosan)	WA = 23 g/g Prolonged release ≅ 50% in first day	Leon et al., 2018
Chitosan - humic material – urea - sodium tripolyphosphate microspheres	Burst effect ≅ 85% in first 4 h Prolonged release ≅ 100 % after 1 day	Araújo, Romão, Doumer & Mangrich, 2017
Urea encapsulated in chitosan microspheres crosslinked with genipin	WA = 1.64 g/g Burst effect ≅ 45% in first 2 days Prolonged release ≅ 90% after 7 days	Hussain, Devi & Maji, 2012
Urea adsorbed into silk fibroin-gelatin-chitosan hydrogels	WA = 4.2 g/g Prolonged release ≅ 80% after 10 days	Rattanamanee, et al., 2015

3.6 Practical application of the formulations

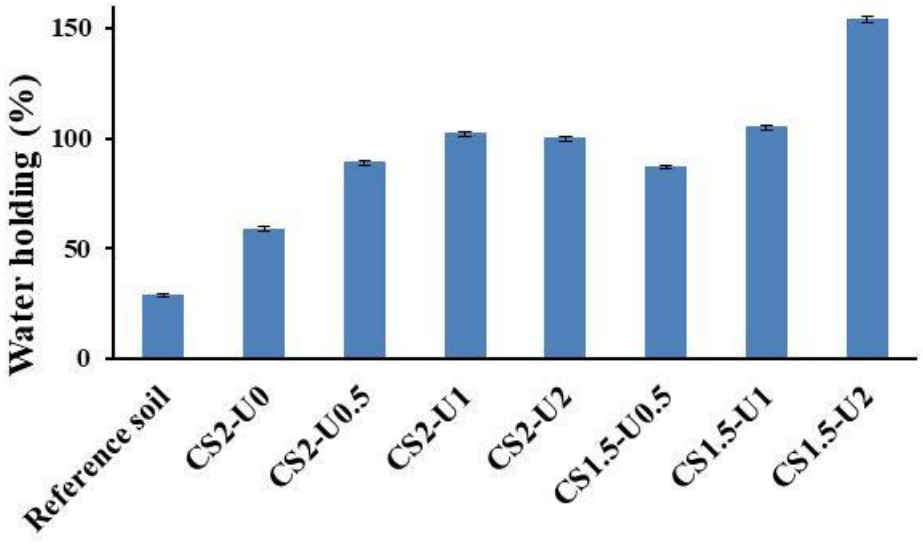
To have a preliminary insight on the ability of the designed formulations to act as soil conditioners, their ability to fertilize the soil and to hold the water were investigated on **CS2-U0.5** and **CS2-U1** samples, by measuring the largest water-holding ratio and the nitrogen dynamic in soil, and the morphological parameters of tomato seedlings in germination experiments (Figure 6 and Table S9 in Supporting Information).

An outstanding increase of the largest water holding parameter, from 29 for the reference soil to 154 wt.% for the **CS1.5-U2** formulation has been recorded, as can be seen in Figure 6a.

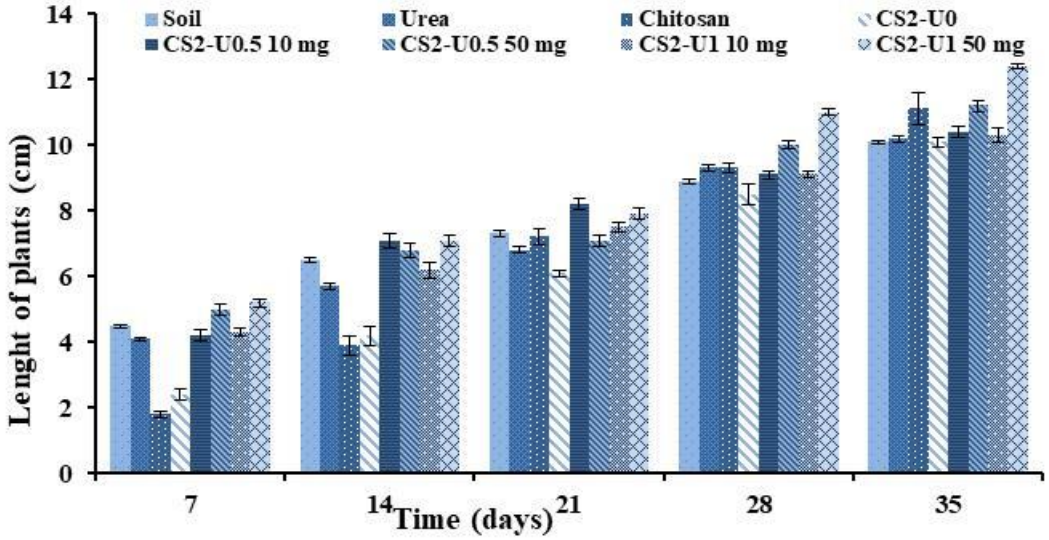
Moreover, the soil sample without xerogels lost the absorbed water after 7 days while those with formulations after 12 days. These results confirmed the expectations, demonstrating that they can improve the water-holding capacity of soil and could obviously reduce the water evaporation.

The evaluation of the dynamic of the nitrogen in soil revealed highest values for the experimental variants including the studied formulations, reaching almost double values (up to 2.07% compared to 0.87% in the blank soil, see Table S9), highlighting the favorable role of the sustained urea release.

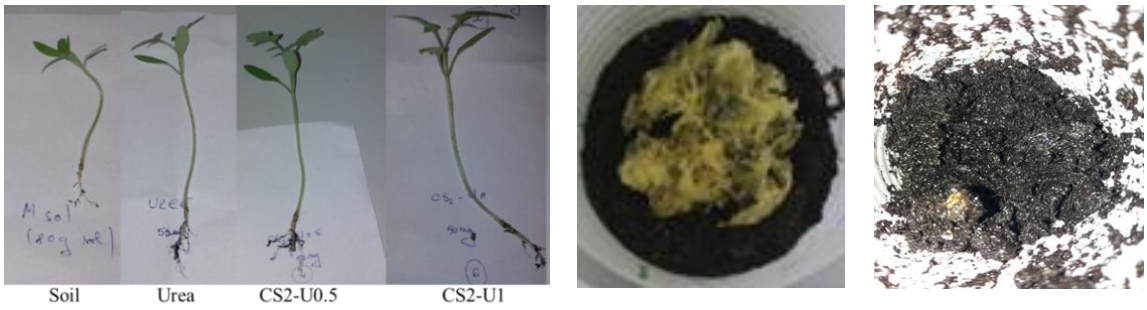
The measurements of the morphological parameters of tomato seedlings (green root weight (FW); dry root weight (DW) and length of plants (PL)) indicated the positive influence of the formulations on the plant growth, as can be seen in Table S9 and Figure 6. These results showed that the formulations stimulated the plant growth up to almost 70% higher than the reference soil. They were supported by the increment of the total content of nitrogen in the fertilized soil with 138% compared to the blank soil. Moreover the formulations significantly reduced their dimension during experiments indicating their biodegradability, as can be seen in Figure 6c,d, while the increment of the morphological parameters for the plants germinated in the soil conditioned with chitosan and CS2-U0 indicated the fertilizing effect of their biodegradation products (see Table S9 and Figure 6). Considering the enriching of the soil with nitrogen and the higher growth indexes obtained when reference hydrogels samples were used, it can be estimated that the degradation products of the hydrogel matrix have no nocive effect and moreover they have a fertilizing effect, as estimated.



a)



b)



c) d) e)

Fig. 6. Graphical representation of a) the largest water-holding ratio of the soil fertilized with **CS2-Ux** and **CS1.5-Ux** formulations; b) length of tomato seedlings function of time; c) Photographs of the tomato seedlings at the end of the experiment; Photographs of the formulation sample in soil, before (d) and after (e) seedling growth experiment

4. Conclusions

New systems designed as soil conditioners were prepared by *in situ* hydrogelation of chitosan with salicylaldehyde in the presence of urea. They were formulated to have different crosslinking density and urea content, in order to optimize the best compositions for the targeted application. The collected data from FTIR spectra and X-ray diffraction revealed that urea has been encapsulated mostly as submicrometric crystals anchored into the hydrogel matrix by H-bonds with chitosan. SEM images showed that the formulations were porous and urea crystals were embedded into the pore walls. *In vitro* release investigation evidenced that urea was delivered in three stages governed by the morphological changes of the hydrogel matrix and anchoring forces: (1) a burst release during first 5 hours controlled by a non-Fickian diffusion through the matrix in the swelling transition; (2) a prolonged release in the next 11 days controlled by a

Fickian diffusion through the swollen matrix; and (3) the release of the remanent urea up to the day 35. Preliminary investigation of the samples as soil fertilizers suggested benefits of their use, by improving the water-holding capacity and nitrogen percentage of the soil, while being biodegradable. All these findings indicate the new systems as multifunctional soil conditioners, opening up new perspectives in the design of eco-materials suitable for sustainable agriculture.

Acknowledgements

The research leading to these results has received funding from the Romanian National Authority for Scientific Research, MEN-UEFISCDI grant, project number PN-III-P1-1.2-PCCDI-2017-0569, Contract nr. 10PCCDI/2018.

References

Abobatta, W. (2018). Impact of hydrogel polymer in agricultural sector. *Advances in Agriculture and Environmental Science: Open Access, 1(2),* 59-64.

Agehara, S., Warncke, D.D. (2005). Soil Moisture and Temperature Effects on Nitrogen Release from Organic Nitrogen Sources. *Soil Science Society of America Journal*, 69, 1844-1855.

Ailincai, D., Marin, L., Morariu, S., Mares, M., Bostanaru, A.C., Pinteala, M., Simionescu, B.C., Barboiu, M. (2016). Dual crosslinked iminoboronate-chitosan hydrogels with strong antifungal activity against Candida planktonic yeasts and biofilms. *Carbohydrate Polymers, 152,* 306-316.

Araújo, B.R., Romão, L.P.C., Doumer, M.E., Mangrich, A.S. (2017). Evaluation of the interactions between chitosan and humics in media for the controlled release of nitrogen fertilizer. *Journal of Environmental Management*, 190, 122-131.

Araújo, B.R., Romaoa, L.P.C., Doumer, M.E., Mangrich, A.S. (2017). Evaluation of the interactions between chitosan and humics in media for the controlled release of nitrogen fertilizer. *Journal of Environmental Management, 190,* 122-131.

Azeem, B., KuShaari, K.Z., Man, Z.B., Basit, A., Thanh, T.H. (2014). Review on materials & methods to produce controlled release coated urea fertilizer. *Journal of Controlled Release, 181,* 11-21.

Bejan, A., Ailincai, D., Simionescu, B.C., Marin, L. (2018). Chitosan hydrogelation with a phenothiazine based aldehyde: a synthetic approach toward highly luminescent biomaterials. *Polymer Chemistry, 9,* 2359-2369.

Campos, E.V.R., Oliveira, J.Z. Fraceto, L.F., Singh, B. (2015). Polysaccharides as saffer release systems for agrochemicals. *Agronomy for Sustainable Development, 35,* 47-66.

Corradini, E., Moura, M.R. de, Mattoso, L.H.C. (2010). A preliminary study of the incorparation of NPK fertilizer into chitosan nanoparticles. *eXPRESS Polymer Letters, 4*, 509–515.

Craciun, A.M., Mititelu-Tartau, L., Pinteala, M., Marin, L. (2019). Nitrosalicyl-imine-chitosan hydrogels based drug delivery systems for long term sustained release in local therapy. *Journal of Colloid and Interface Science*, *536*, 196-207.

Gatti, C., Saunders, V.R., Roetti, C. (1994). Crystal field effects on the topological properties of the electron density in molecular crystals: the case of urea. *The Journal of Chemical Physics*,101, 10242.

Godoy-Alcántar, C., Yatsimirsky, A.K., Lehn, J.-M. (2005). Structure-stability correlations for imine formation in aqueous solution. *Journal of Physical Organic Chemistry*, 18, 979-985.

Guilherme, M.R., Aouada, F.A., Fajardo, A.R., Martins, A.F., Paulino, A.T., Davi, M.F.T., Rubira, A.F., Muniza, E.C. (2015). Superabsorbent hydrogels based on polysaccharides for application in agriculture as soil conditioner and nutrient carrier: a review. *European Polymer Journal*, *72*, 365–385.

Hargreaves, J.C., Adl, M.S., Warman, P.R. (2008). A review of the use of composted municipal solid waste in agriculture. *Agriculture Ecosystems & Environment*, *123*, 1-14.

Hussain, M.R., Devi, R.R., Maji, T.K. (2012). Controlled release of urea from chitosan microspheres prepared by emulsification and cross-linking method. *Iranian Polymer Journal*, 21, 473-479.

Iftime, M.M., Marin, L. (2018). Chiral betulin-imino-chitosan hydrogels by dynamic covalent sonochemistry. *Ultrasonics Sonochemistry*, *45*, 238-247.

Iftime, M.M., Morariu, S., Marin, L. (2017). Salicyl-imine-chitosan hydrogels: Supramolecular architecturing as a crosslinking method toward multifunctional hydrogels. *Carbohydrate Polymers*, *165*, 39-50.

Iriti, M., Varoni, E.M. (2015). Chitosan-induced antiviral activity and innate immunity in plants. *Environmental Science and Pollution Research*, *22*, 2935-2944.

Kato, S., Kishiro, F., Yokohagi, O., Vijayanand, P.S., Satokawa, S., Kojima, T. (2017). Synthesis of novel biodegradable superabsorbent polymer using chitosan for desert land development. *Journal of Arid Land Studies*, *27*, 67-74.

Leon, O., Munoz-Bonilla, A., Soto, D., Ramirez, J., Marquez, Y., Colina, M., Fernandez-Garcia, M. (2018). Preparation of Oxidized and Grafted Chitosan Superabsorbents for Urea Delivery. *Journal of Polymers and the Environment*, 26, 728-739.

Lin, C.C., Metters, A.T. (2006). Hydrogels in controlled release formulations: Network design and mathematical modeling. *Advanced Drug Delivery Reviews*, *58*, 1379-1408.

Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J.W., Goulding, K., Christie, P., Fangmeier, A., Zhang, F. (2013). Enhanced nitrogen deposition over China. *Nature*, *494*, 459–462.

Madhurambal, G., Mariappan, M., Mojumdar, S.C. (2010). Thermal, UV and FTIR spectral studies of urea–thiourea zinc chloride single crystal. *Journal of Thermal Analysis and Calorimetry*, *100*, 763–768.

Majeed, Z., Ramli, N.K., Mansor, N., Man, Z. (2015). A comprehensive review on biodegradable polymers and their blends used in controlled-release fertilizer processes. *Reviews in Chemical Engineering*, *31*, 69-96.

Malerba, M., Cerana, R. (2016). Chitosan effects on plant systems. *International Journal of Molecular Sciences*, *17*, E996.

Marin, L., Ailincai, D., Morariu, S., Tartau-Mititelu, L. (2017). Development of biocompatible glycodynameric hydrogels joining two natural motifs by dynamic constitutional chemistry. *Carbohydrate Polymers*, *170*, 60-71.

Marin, L., Popescu, M.C., Zabulica, A., Uji-I, H., Fron, E. (2013). Chitosan as a matrix for biopolymer dispersed liquid crystal systems. *Carbohydrate Polymers*, *95*, 16-24.

Narayanan, A., Dhamodharan, R. (2015). Super water-absorbing new material from chitosan, EDTA and urea, *Carbohydrate Polymers*, *134*, 337-343.

Naz, M.Y., Sulaiman, S.A. (2016). Slow release coating remedy for nitrogen loss from conventional urea: a review. *Journal of Controlled Release*, *225*, 109-120.

Ni, B., Liu, M., Lü, S. (2009). Multifunctional slow-release urea fertilizer from ethylcellulose and superabsorbent coated formulations. *Chemical Engineering Journal*, *155*, 892–898.

Olaru, A.M., Marin, L., Morariu, S., Pricope, G., Pinteala, M., Tartau-Mititelu, L. (2018). Biocompatible chitosan based hydrogels for potential application in local tumour therapy. *Carbohydrate Polymers*, *179*, 59-70.

Perez, J.J., Francois, N.J. (2016). Chitosan-starch beads prepared by ionotropic gelation as potential matrices for controlled release of fertilizers. *Carbohydrate Polymers*, *148*, 134-142.

Pichyangkura, R., Chadchawanb, S. (2015). Biostimulant activity of chitosan in horticulture. *Scientia Horticulturae*, *196*, 49–65.

Rattanamanee, A., Niamsup, H., Srisombat, L., Punyodom, W., Watanesk, R., Watanesk, S. (2015). Role of chitosan on some physical properties and the urea controlled release of the silk fibroin/gelatin hydrogel. *Journal of Polymers and the Environment*, 23, 334-340.

Samoila, P., Sacarescu, L., Borhan, A.I., Timpu, D., Grigoras, M., Lupu, N., Zaltariov, M., Harabagiu, V. (2015). Magnetic properties of nanosized Gd doped Ni–Mn–Cr ferrites prepared

using the sol–gel autocombustion technique. *Journal of Magnetism and Magnetic Materials, 378*, 92–97.

Santos, B.R., Bacalhau, F.B., Pereira, T.S., Souza, C.F., Faez, R. (2015). Chitosan-Montmorillonite microspheres: a sustainable fertilizer delivery system. *Carbohydrate Polymers, 127,* 340-346.

Tao, Y., Liu, S., Zhang Y., Chi, Z., Xu, J. (2018). pH-responsive polymer based on dynamic imine bonds as a drug delivery material with pseudo target release behavior. *Polymer Chemistry*, *9*, 878-884.

Wu, L., Liu, M.Z. (2008). Preparation and properties of chitosan-coated NPK compound fertilizer with controlled-release and water-retention. *Carbohydrate Polymers, 72*, 240-247.

Yang, L., An, D., Wang, T.J., Kan, C.Y., Jin, Y. (2017). Photodegradation of polymer materials used for film coatings of controlled-release fertilizers. *Chemical Engineering & Technology, 40*, 1611-1618.

Zhao, C., Shen, Y.Z., Du, C.W., Zhou, J. M., Wang, H.Y., Chen, X.Y. (2010). Evaluation of waterborne coating for controlled-release fertilizer using wurster fluidized bed. *Industrial & Engineering Chemistry Research*, *49*, 9644-9647.