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Soluble vitamins (vitamin B12 and vitamin C) microencapsulated with different biopolymers by a spray drying process

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Abstract

Vitamins are important micro nutritional compounds which are involved in many biochemical functions in the Human body but are not synthesized by it; so, they have to be supplied through diet. However, vitamins are very sensitive which provoke a significant loss during the food processes and storage. So, microencapsulation can be used to minimize the loss of vitamins, to minimize the factors that interfere with their stability, to allow a controlled release process and to mask its undesirable taste, increasing their applicability.

In the present work, the microencapsulation of two vitamins, by a spray-drying process, was studied: vitamin B12, considering that is the most chemically complex and the largest of all the vitamins and vitamin C which is the most popular vitamin in the food industry. The microparticles were prepared using a spray-dryer BÜCHI B-290 (Flawil, Switzerland) with a standard 0.5 mm nozzle, under the following conditions: solution and air flow rates, air pressure and inlet temperature were set at 4 ml/min (15%), 32 m3/h (80%), 6.0 bar and 120 ºC, respectively. The prepared microparticles were characterized and their physicochemical structures were analyzed by scanning electron microscopy (SEM) and by Fourier transform infrared spectroscopy (FTIR). The presence of vitamins in the microparticles was also evaluated by UV- method, validated and optimized for this objective. The evaluation of the vitamin B12 was based on absorbance values read at 361.4 nm, and for the vitamin C the absorbance was read at 260.6 nm.

A product yield ranging from 41.8 to 55.6% for the microparticles prepared with vitamin B12 and ranging from 43.6 to 45.4% for the microparticles formed with vitamin C was obtained and microparticles with a mean diameter around 3 μm were observed, for all the biopolymers tested (chitosan, modified chitosan and sodium alginate). The microparticles formed with chitosan presented a very rough surface; on the other hand, the particles

formed with sodium alginate or modified chitosan presented a very smooth surface. The performed tests yield significant results and prove the success of the vitamins microencapsulation.

This work shows that it is possible to encapsulate vitamins using different biopolymers, through a spray-drying process.

Keywords:

Encapsulating Agent, Microencapsulation, Microcapsules, Spray Drying, Vitamin C, Vitamin B12.

1. Introduction

Vitamins are bioactive compounds; in food are physiologically active components that provide health benefits beyond their nutritional role [1]. Vitamins are important micro nutritional substances involved in many biochemical functions in the Human body, but they are not synthesized by it; so, they have to be supplied through diet [2]. One diet poor in vitamins can lead to many deficiency diseases like pernicious anemia, scurvy, pellagra, ariboflavinosis, dermatitis, enteritis, among others. This research is focused in two main water soluble vitamins: vitamin B12 and vitamin C.

Vitamin B12 is also called cobalamin because it has cobalt in this structure and is the most chemically complex and largest of all the vitamins. Vitamin B12 belongs to the group "corrinoids," which is a group of compounds having a corrin macrocycle. Vitamin B12 has a molecular weight of 1355.4, is stable in aqueous solution of pH 4−7 and can be heated at 120 °C without significant losses [3,4]. Vitamin B12 is involved in the cell metabolism (DNA synthesis and regulation), in the normal operation of the brain and nervous system, and in the formation of blood. Vitamin B12 is produced by certain bacteria and is concentrated in the bodies of higher predators in the food chain. Therefore, foods derived from animals are considered to be the major dietary sources of B12 [3,4]. People with a limited intake of food with an animal source have a high risk of suffering of B12 deficiency. Vitamin B12 is related to some diseases, like the pernicious anemia. One solution is to consume vitamin B12-fortified foods or vitamin B12-containing dietary supplements to prevent B12 deficiency [3,4]. In addition low values of vitamin B12 were reported in animals, and one of the solutions is the incorporation of vitamin B12 in additives [5–7].

Vitamin C is a well-known bioactive compound and is a representative water soluble vitamin. Vitamin C has a variety of biological, pharmaceutical and dermatological

functions. [8] Vitamin C helps in fighting common colds by strengthening the immune system and is important for its potential role in minimizing the risk of serious diseases such as cancer, heart disease, cataracts, and high lead levels. Deficiency in vitamin C is associated with the disease known as scurvy [9]. Unfortunately, the human body is unable to synthesize vitamin C and cannot store it. So, appropriate amounts must be supplied regularly through the diet to restock this valuable compound [9]. Vitamin C is widely used in various types of foods as a vitamin supplement. However, vitamin C is very unstable to air, moisture, light, heat, oxygen and alkaline pH and easily decomposes into biologically inactive compounds [8]. Furthermore, due to its acidic nature, it can interact with other food components and thus negatively affect the sensory properties and shelf life of vitamin C-fortified foods [10].

Therefore, microencapsulation could be used as an alternative to minimize the factors that interfere with the stability of the vitamins, allow for the controlled release and mask its undesirable taste, which can be unpleasant [8,11]. It is important to microencapsulate these vitamins in order to increase their applicability in food processes. For example, some authors studied the possibility of microencapsulating vitamins with the purpose of increasing their resistance to the cooking process or storage [9]. Different studies it concluded that, Vitamin C, Vitamin B9 and vitamin B6 are less stable during high-temperature processing as compared to retinol, thiamine, riboflavin and niacin. Almost all these studies were made with encapsulating agents or processes that request the presence of organic solvents that can increase the toxicity of the particles produced. New solutions and techniques are requested. Borrmann et al. (2013) microencapsulated passion fruit juice (Vitamin C) with n-octenyl succinate-derivatised starch using a spray-dryer and stored at two different temperatures. Bastos et al. (2012) microencapsulatated cashew apple (*Anacardium occidentale,L.*) juice (Vitamin C) using also a spray-drying process.

106 The spray-drying process is flexible and produces microparticles of good quality and is
107 also, a relatively low cost technology, rapid, reproducible, allowing easy scale-up, when
108 compared with other microencapsulation techniques, justifying the preference in
109 industrial terms [2,14–20].
110 The present work shows the recent developments and the new applications of the spray
111 drying technology for microencapsulation of two different vitamins (vitamin B12 and
112 vitamin C) with different biopolymers: chitosan, modified chitosan and sodium alginate,
113 considering all the advantages of these biopolymers. On the other hand, the two selected
114 vitamins were chosen considering their complexity (vitamin B12) and their high
115 applicability (vitamin C).
116

117 2. Material and Methods

118 2.1. Preparation of the solutions

119 Microparticles of two soluble vitamins (vitamin C and vitamin B12) were prepared. The
120 vitamin C was a reference standard of ascorbic acid (Cat. No. 1043003, Lot ROK 142)
121 from USP Rockville, MD (USA). The Vitamin B12 (Cat. No. V2876, Lot #
122 MKBQ9972V) with a purity ≥ 98% was from Sigma-Aldrich (China). Solutions of these
123 two vitamins were prepared with concentrations of 10 g/L using deionised water and
124 agitation at 1200 rpm.
125 Three different biopolymers were used to prepare microparticles with vitamins: chitosan,
126 a modified chitosan (water soluble) and sodium alginate. Chitosan of medium molecular
127 weight (Cat. No. 448877) was purchased from Aldrich (Germany). The solution of
128 chitosan at 1% (w/V), prepared in a solution of acetic acid (1% (V/V)) has a viscosity of
129 200 mPa.s (25 ºC). Water soluble chitosan (pharmaceutical grade) was obtained from
130 China Eastar Group (Dong Chen) Co., Ltd (Batch no. SH20091010). Water soluble

chitosan was produced by carboxylation and had a deacetylation degree of 96.5%. The solution of modified chitosan at 1% (w/V), prepared in deionised water has a viscosity of 5mPa.s (25 ºC). Sodium alginate (alginic acid, sodium salt) (Cat. No. 180947) was from Aldrich (USA).

All the three solutions were prepared at room temperature. The chitosan solution was prepared with a concentration of 1% (w/V) in an acetic acid solution 1% (V/V) and with 2 hours agitation at 1200 rpm (magnetic agitator – MS-H-Pro, Scansci). The other two solutions, of water soluble chitosan 1% (w/V) and sodium alginate 1% (w/V) were prepared with deionised water and with 2 hours agitation at 1200 rpm.

To obtain the vitamin microparticles it was necessary to prepare solutions containing the vitamins and the encapsulating agents. These solutions were then fed to the spray dryer. Thus, the solution containing the vitamin was added and mixed with each one of the biopolymers aqueous solutions (encapsulating agents) at constant agitation speed of 1200 rpm, during 10 min at room temperature. The concentration of the vitamin in the fed solution to the spray-dryer was 2.0 % (w/w). Also, microparticles without vitamin were prepared, in order to study the effect of the vitamin on the microparticles produced, under the same conditions.

2.2. Experimental conditions – Spray-drying process

Spray-drying was performed using a spray-dryer BÜCHI B-290 (Flawil, Switzerland) with a standard 0.5 mm nozzle. The same procedure was followed for all the microparticles prepared with vitamin B12 and vitamin C and also for microparticles prepared without vitamin. The solutions were spray-dried, under the following conditions: solution and air flow rates, air pressure and inlet temperature were set at 4 ml/min (15%), 32 m^3/h (80%), 6.0 bar and 120 ºC, respectively. The outlet temperature,

a consequence of the other experimental conditions and of the solution properties, was around 65 ºC. The operating conditions have been selected considering preliminary studies. All the experiments were made in duplicated, with a coefficient of variation smaller than 10%.

2.3. SEM characterization

Structural analysis of the surface of the particles was performed by scanning electron microscopy (Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M). The surface structure of the particles was observed by SEM after sample preparation by pulverization of gold in a Jeol JFC 100 apparatus at Centro de Materiais da Universidade do Porto (CEMUP).

2.4 FTIR analysis

The chemical characterization of microparticles was performed by Fourier transform infrared spectroscopy (FTIR) in a Bomem–MB Series, Arid-ZoneTM (Québec, Canada). The spectra were obtained with KBr at 99%, at 21 scans/min, with a resolution of 4 cm^{-1} and expressed in transmittance in the 4000−650 cm^{-1} range.

2.5. Evaluation of the presence of vitamins in the microparticles

The evaluation of the presence of the vitamin B12 and vitamin C in the microparticles was made by an UV method. Two calibration curves, one for each vitamin, were developed to evaluate the concentration of vitamins released to the solution.

Small amounts (3 mg) of powder containing the vitamin microparticles were added to 3 ml of deionised water; the maximum concentration of released vitamin was estimated by mass balance, considering the amount of reagents used, proportions vitamin/biopolymer and specifications of the spray drying process, and was also determined experimentally

considering the amount of vitamin released from the microparticles. The released vitamin was determined in continuous absorbance measurements (intervals of 30 seconds) until the maximum value of the released vitamin was obtained and stabilized.

The determination of the presence of the vitamin B12 and vitamin C in the microparticles was based on absorbance values, read at room temperature in an UV-Visible spectrophotometer (SCANSPEC SP110070 from SCANSCI) at 361.4 nm and 260.6 nm respectively.

Two calibration curves were developed to evaluate the concentration of vitamin released to the solution. The calibrations were made in duplicated with coefficients of variation smaller than 10% for all the standards.

For vitamin B12, standard solutions were prepared in deionised water. The determination of vitamin B12 was validated in the concentration range of 0.0025 g/L to 0.1 g/L with 12 standards and with a correlation coefficient of the method of 0.985. The detection limit determined for vitamin B12 was 0.006 g/L.

For the vitamin C, the calibration was developed and validated in the concentration range of 0.0005 g/L to 0.022 g/L with 10 standards (prepared in deionised water), with a correlation coefficient of 0.985 and a detection limit of 0.001 g/L.

3. Results and Discussion

The spray drying process was performed with previously fixed operating conditions, in order to compare the microcapsules formed with different encapsulating agents (chitosan, modified chitosan and sodium alginate).

The product yield (quantity of powder recovered reported to the quantity of raw materials used) for the microparticles with vitamin B12 was 41.8%, 55.6% and 42.4% when prepared with sodium alginate, chitosan and modified chitosan, respectively. In the case

206 of the microparticles with vitamin C a product yield of 43.6%, 44.5% and 45.4%,
207 respectively, was obtained. The product yields obtained for these microparticles are very
208 similar with the ones obtained by Estevinho et al (2014) [21] to prepare β-galactosidase
209 microparticles with the same encapsulating agents. Estevinho et al (2015) [22] also
210 discussed the existence of small product yields, around 30-50%, for the
211 microencapsulation by a spray drying technique. When the inlet temperature is lower, as
212 in the present case (120 ◦C), the probability of obtaining low product yields increases. At
213 low temperatures, the deposition of particles on the cylinder or/and on the cyclone wall
214 of spray dryer was observed, leading to a lower product yield. On the other hand, the
215 particles formed by this method are very small (around 3 μm), and the efficiency of the
216 cyclone to separate small particles decreases, some of them being aspirated with the air
217 leaving the spray dryer. Also, the sample volume influences the product yield; small
218 volumes implying higher relative losses [22].
219 The prepared microparticles were characterized and their physicochemical structures
220 were analyzed by SEM and by FTIR.
221

222 ## 3.1. Microparticles characterization

223 ### 3.1.1. Scanning electron microscopy (SEM) analysis

224 Spherical microparticles were produced in all the cases (Fig. 1). The surface of the
225 microparticles presented different textural characteristics. In the case of the particles
226 formed with chitosan the surface was very rough. The particles formed with sodium
227 alginate had a smooth surface and the microparticles formed with modified chitosan
228 presented a very regular shape and a smooth surface. Microparticles with a mean diameter
229 around 3 μm were observed, for all the biopolymers tested (chitosan, modified chitosan
230 and sodium alginate). In SEM images, the size of the microparticles containing vitamins

appears to be similar to the size of the microparticles produced without vitamins. Estevinho et al (2014) [23] found similar results, surface, textural characteristics and size, for the microencapsulation of β-galactosidase with the same encapsulating agents. Also the microparticles size without enzyme appears to be similar to the size of the microparticles with enzyme.

3.1.2. FTIR analysis

FTIR studies give information about the molecular structure of chemical compounds and are useful for the characterization of biopolymers.

In this work, 3 different biopolymers (sodium alginate, chitosan and modified chitosan) were tested, taking into account their high biocompatibility. In Fig. 2, it is possible to evaluate the FTIR spectrum of the microparticles made with these biopolymers. All of them are polysaccharides with similar functional groups, that will provoke a high similarity between the spectra.

Alginate is a natural, linear, unbranched polysaccharide containing 1,4´-linked beta-D-mannuronic and alpha-L-guluronic acid residues [24]. For sodium alginate microparticles the more important absorption bands at frequency values that justify the existence of the corresponding functional groups (bonds), are for example: O-H (3700-3000 cm^{-1}; stretch vibration), C-H (3000-2850 cm^{-1}; stretch vibration), CO_2- (1600 cm^{-1} antisymmetric CO_2-stretch), CO_2- (1400 cm^{-1} symmetric CO_2-stretch), at 1300 cm^{-1} skeletal vibration and at 1100-1000 cm^{-1} antisymmetric stretch C-O-C. These bands are consistent with the results of, for example, Lawrie et al. (2007) [25].

Chitosan is an attractive biopolymer, although a water-insoluble material; however it is possible to modify the structure in order to produce an easily soluble chitosan in neutral

255 aqueous solutions [26–28]. Water soluble chitosan can be useful for drug carriers and for
256 food industrial applications [19,29].
257 For chitosan microparticles the more important absorption bands are at frequency values:
258 3700-3000 cm^{-1} (O-H and N-H, stretch vibration), 3000-2850 cm^{-1} (C-H, stretch
259 vibration), 1645 cm^{-1} (Amide I), 1584 cm^{-1} (N-H bending from amine and amine II), 1410
260 cm^{-1} (-CH_2 bending), 1375 cm^{-1} (CH_3 symmetrical deformation), 1150 cm^{-1}
261 antisymmetric stretch C-O-C and C-N stretch and at 1030 skeletal vibration of C-O
262 stretching. The band at 1560 cm^{-1} has a larger intensity than at 1655 cm^{-1}, which suggests
263 effective deacetylation of chitosan. The band at 1656 cm^{-1} corresponds to the amide I
264 stretching of C=O, as described by Lawrie *et al.* (2007) [25,30]. The spectrum of the
265 microparticles with modified chitosan is similar to the spectrum of the chitosan; only the
266 size of some absorption bands is different.
267 In Fig. 3, the spectra of the microparticles with vitamin B12 and vitamin C are presented.
268 These microparticles have only 2% of vitamin and 98% of encapsulating agent, which
269 makes it difficult to identify the presence of the vitamins in the spectra, when compared
270 with the spectra of the microparticles made only with the encapsulating agent. Some
271 important absorption bands of the vitamins are overlapped by the absorption bands of the
272 encapsulating agent making it difficult to distinguish them.
273 The major band for the vitamin B12 occurs at frequency values of 1664 cm^{-1} and is due
274 to the amide I C=O stretching mode of the propionamide side chains of the corrin ring
275 [31]. These authors also describe a medium intensity band at 1572 cm^{-1}, attributed to a
276 breathing mode of the corrin ring of vitamin B12 and another one at a frequency 2135
277 cm^{-1} (cyanide stretching), proving the cobalt-carbon distance in cyanocorrinoids of
278 vitamin B12 [31].

279 The strongest absorption bands for the vitamin C occur at frequency values: 1764 cm^{-1}
280 (C=O stretching), 1675 cm^{-1} (C=C ring stretching) and 3216–3626 cm^{-1} (OH stretching).
281 Various vibrational bands can be observed in the region 1200–1500 cm^{-1} which are
282 connected with the CH_2 scissoring, twisting and wagging and the C-H deformation
283 modes. The band at 1277 cm^{-1} is originated by C-O-C stretching. Others C-O-C stretches
284 can be seen at 1142, 1121, 1113, 1077 and 1046 cm^{-1} in the vitamin C spectrum [32].
285 In FTIR spectra of the microparticles made with alginate (Fig. 3 A) the presence of the
286 vitamins was very difficult to recognize. There are only small differences in the size or in
287 the proportion of the bands.
288 In the case of chitosan (Fig. 3 B), for the microparticles with vitamin C the bands at
289 frequency values of 1764 cm^{-1}, 1675 cm^{-1} and 1277 cm^{-1} are different and appear to be
290 bigger than in the case of the microparticles made only with chitosan. For the
291 microparticles with vitamin B12 the band at the frequency 2135 cm^{-1} (cyanide stretching)
292 increases, proving the presence of vitamin B12. Finally, for the case of the microparticles
293 with modified chitosan (Fig. 3 C), and for the case of vitamin B12, the bands at frequency
294 values of 1664 cm^{-1} (C=O stretching) and 1572 cm^{-1} (attributed to a breathing mode of
295 the corrin ring) have other size relation than in the spectrum of microparticles made only
296 with modified chitosan. It is also possible to see a small band at a frequency of 2135 cm^{-1}
297 (cyanide stretching). For vitamin C, the bands at frequency values of 1764 cm^{-1} (C=O
298 stretching), 1675 cm^{-1} (C=C ring stretching) and at 1277 cm^{-1} (C-O-C stretching)
299 increased. For both of the cases of the microparticles made with vitamins, the size of the
300 band 3600-3000 cm^{-1} (OH stretching) increased, when compared with the spectrum of
301 the microparticles made only with modified chitosan.
302 The differences between the spectra with and without vitamins are very small, but they
303 give support to the idea that microparticles have vitamins in their composition. To

confirm this, analytical methods have been developed to quantify the presence of vitamins in the microparticles.

3.2. Evaluation of the presence of vitamin B12 and vitamin C in the microparticles

For both of the vitamins the release was total. So, the presence of vitamin B12 and vitamin C in the microparticles was confirmed and also obtained the different release profiles, for the different encapsulating agents. The total amount of the vitamin was recovered in different times depending on the encapsulating agents (Fig. 4 and Fig. 5). For example, for vitamin B12 the total amount of vitamin was released in 120 min for microparticles made with chitosan, in 15 min for microparticles made with alginate and 10 min for the microparticles made with modified chitosan. Similar results have been obtained for the microparticles with vitamin C.

Comparing the SEM images with the release profiles, a slow release was associated to a rougher surface (microparticles with chitosan).

So, depending on the type of application intended for the vitamins, different encapsulating agents need to be selected to allow the more adequate controlled release of the vitamins. For instance if a slow release of the vitamin in one aqueous solution is wanted the best option for the encapsulating agent will be the chitosan. On the other hand if it is intended a fast release of the vitamin the best option can be the microencapsulation of vitamins with modified chitosan or alginate. These two encapsulating agents can be used, for example, for microencapsulated vitamins used in drinks prepared instantaneously from powder formulations. Thus the vitamins will be protected from oxidation, light, moisture and other factors during the storage time.

As referred by Murugesan and Orsat (2012) microencapsulation and nanoencapsulation are the best ways to preserve vitamins [2]. Some authors used with success the spray drying technique to microencapsulate vitamins such as vitamin A [33,34], vitamin E [35] and vitamin C [8]. Vitamin C was successfully encapsulated in tripolyphosphate (TPP) cross-linked chitosan (TPP-chitosan) microspheres by the spray-drying method. The effect of adding a crosslinking agent and how this crosslinking increase the stability of the microparticles was studied [8,36]. The sphericity of chitosan microspheres was lost at higher volume of crosslinking agent. The TPP-chitosan microspheres loaded with vitamin C were spherical and had smooth surface and the release of vitamin C from these microspheres was sustained and affected by the volume of crosslinking agent added. [8,36]. In general the crosslinking agents provoke changes in the structure of the microparticles and delay the release of the compounds from the microparticles, improving the controlled release systems [37]. In the present work, 3 encapsulating agents have been studied and compared, without crosslinking agents. The use of crosslinking agents will be subject of future works.

4. Conclusion

In the present work two different vitamins (vitamin B12 and vitamin C) were microencapsulated by a spray drying process using three different encapsulating agents: chitosan, modified chitosan and sodium alginate.

A product yield around 45% was obtained for both of vitamins in all the assays. Microparticles with a mean diameter around 3 μm were observed, for all the biopolymers tested. The microparticles formed with chitosan presented a very rough surface but the particles formed with sodium alginate or modified chitosan presented a very smooth surface. Finally, the presence of vitamins in the microparticles was confirmed and

353 evaluated by UV-method, validated and optimized for this objective. Different release
354 profiles were obtained for both of the vitamins with the different encapsulating agents
355 (chitosan, modified chitosan and sodium alginate). In general, the release time of the total
356 amount of vitamins was around 120 min for microparticles made with chitosan, 15 min
357 for microparticles made with alginate and 10 min for the microparticles made with
358 modified chitosan.
359 Comparing the SEM images with the release profiles, a slow release was associated to a
360 rougher surface (microparticles with chitosan).
361 This work shows that it is possible to encapsulate vitamins using different biopolymers
362 through a spray-drying process, and depending on the type of application pretended for
363 the vitamins, different encapsulating agents need to be selected to allow the more
364 adequate controlled release of the vitamins.
365

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Figure Captions

Fig. 1: SEM images of the microparticles with vitamins and without vitamins with different biopolymers: sodium alginate, chitosan and modified chitosan. Amplified 30000 times, beam intensity (HV) 1000 kV, distance between the sample and the lens (WD) less than 12 mm.

Fig. 2: FTIR spectra for samples with microparticles made with sodium alginate, chitosan and modified chitosan. Spectra obtained with KBr at 99%, at 21 scans/min, with a resolution of 4 cm^{-1} and expressed in transmittance in the 4000−650 cm^{-1} range.

Fig. 3: FTIR spectra for samples with microparticles with vitamin B12 and vitamin C made with: A - sodium alginate, B - chitosan and C - modified chitosan. Spectra obtained with KBr at 99%, at 21 scans/min, with a resolution of 4 cm−1 and expressed in transmittance in the 4000−650 cm−1 range.

Fig. 4: Release of vitamin B12 from microparticles made with different encapsulating agents.

Fig. 5: Release of vitamin C from microparticles made with different encapsulating agents.

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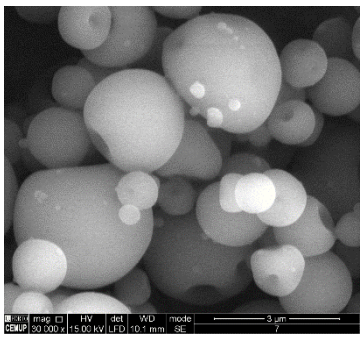
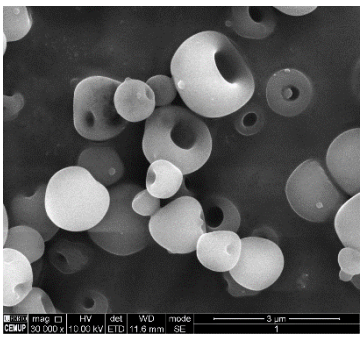
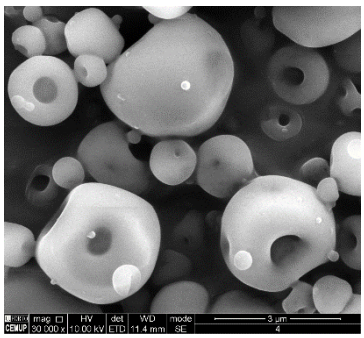
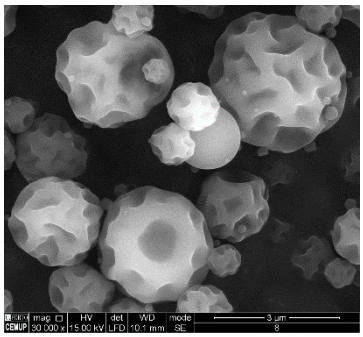
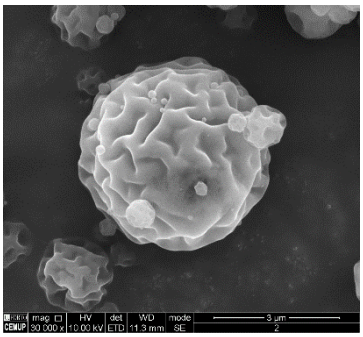
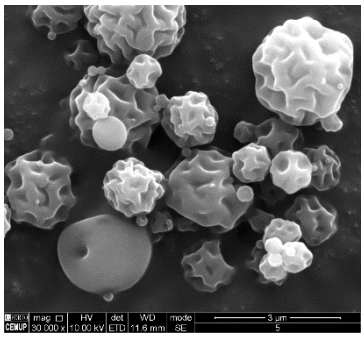
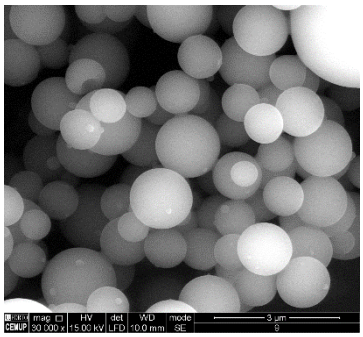
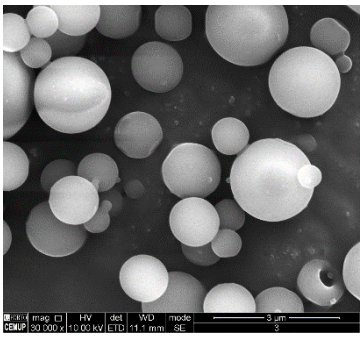
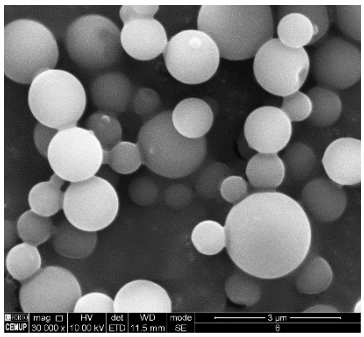
Microcapsules with encapsulating agent	Without Vitamin	Vitamin B12	Vitamin C
Sodium alginate			
Chitosan			
Modified chitosan			

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521 Fig. 1: SEM images of the microparticles with vitamins and without vitamins with
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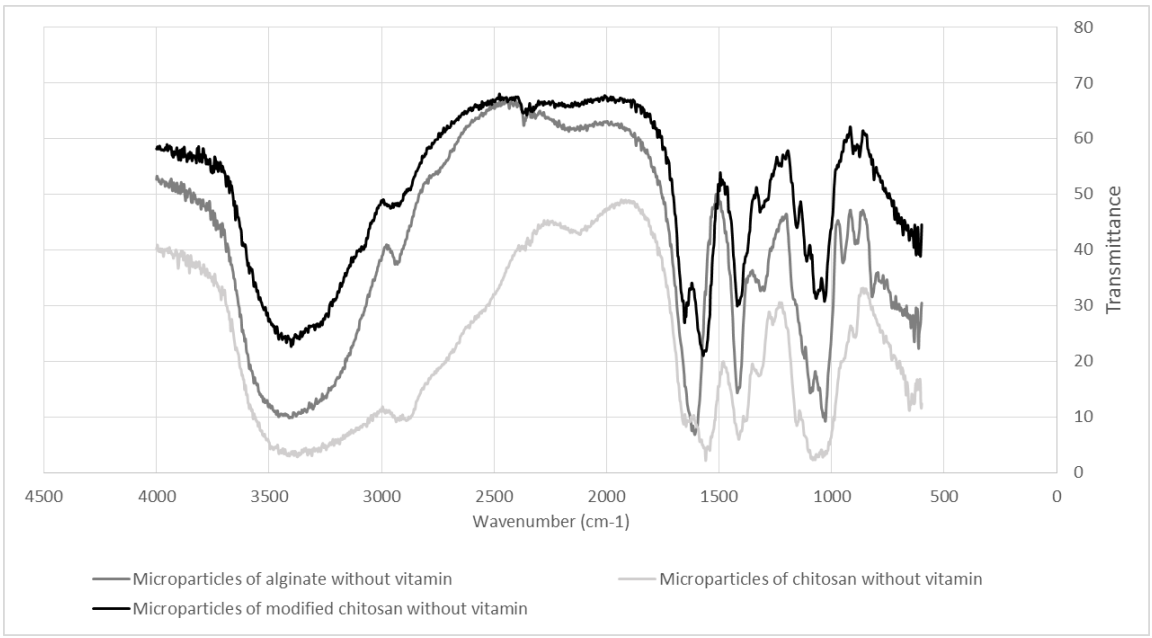

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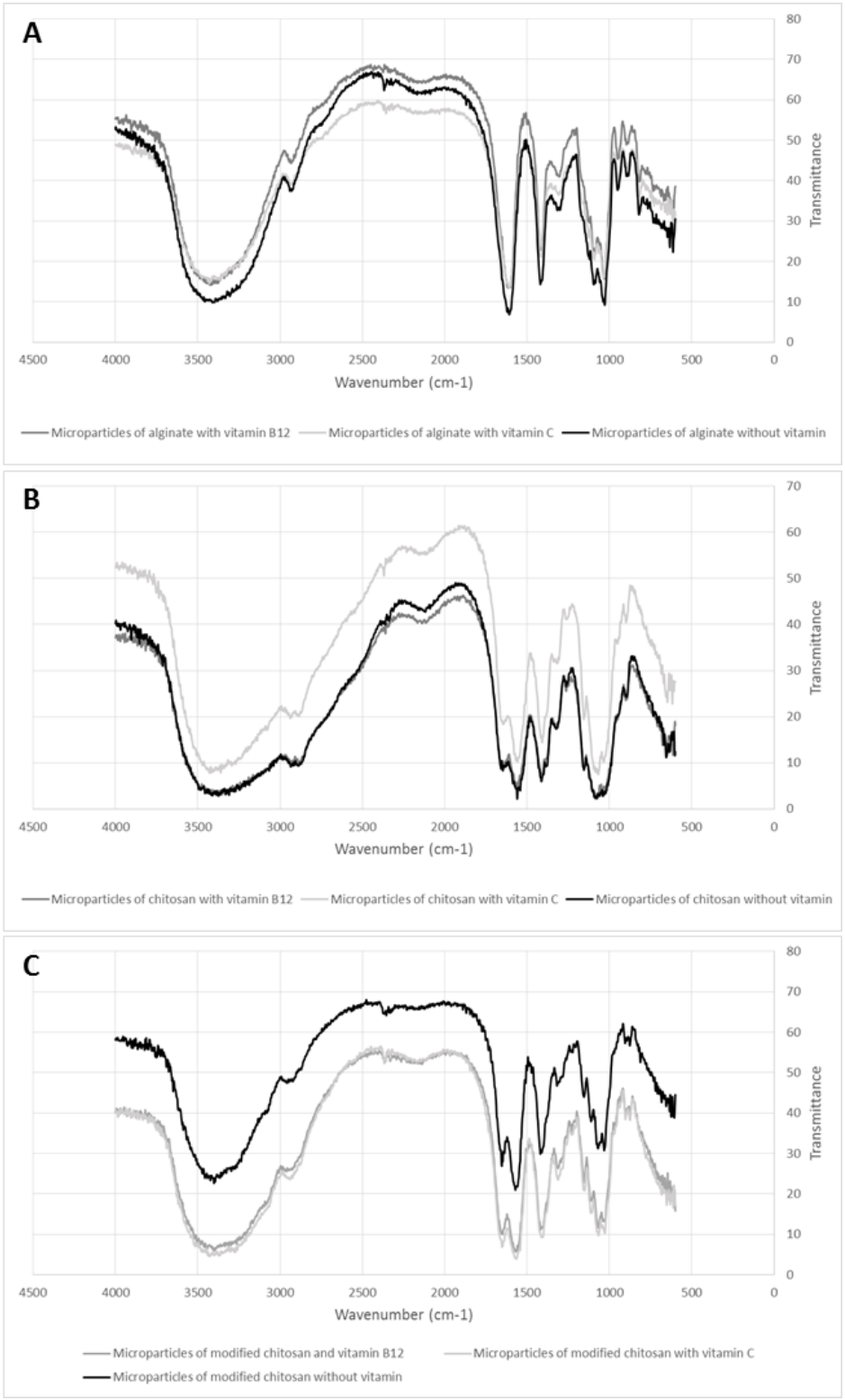

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532 Fig. 3: FTIR spectra for samples with microparticles with vitamin B12 and vitamin C
533 made with: A - sodium alginate, B - chitosan and C - modified chitosan. Spectra obtained
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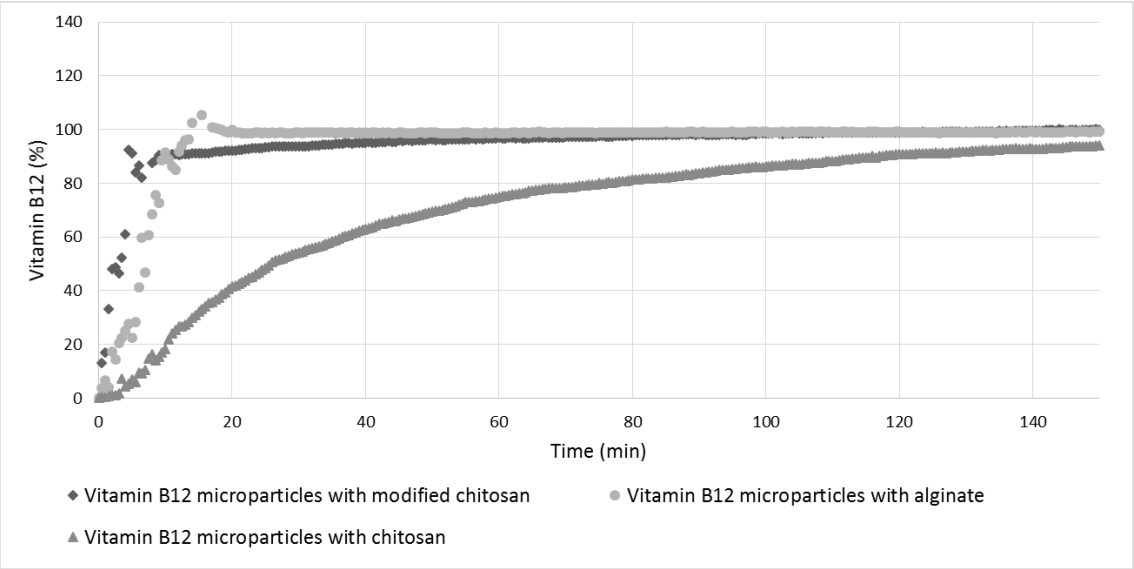

536

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538 agents.

539

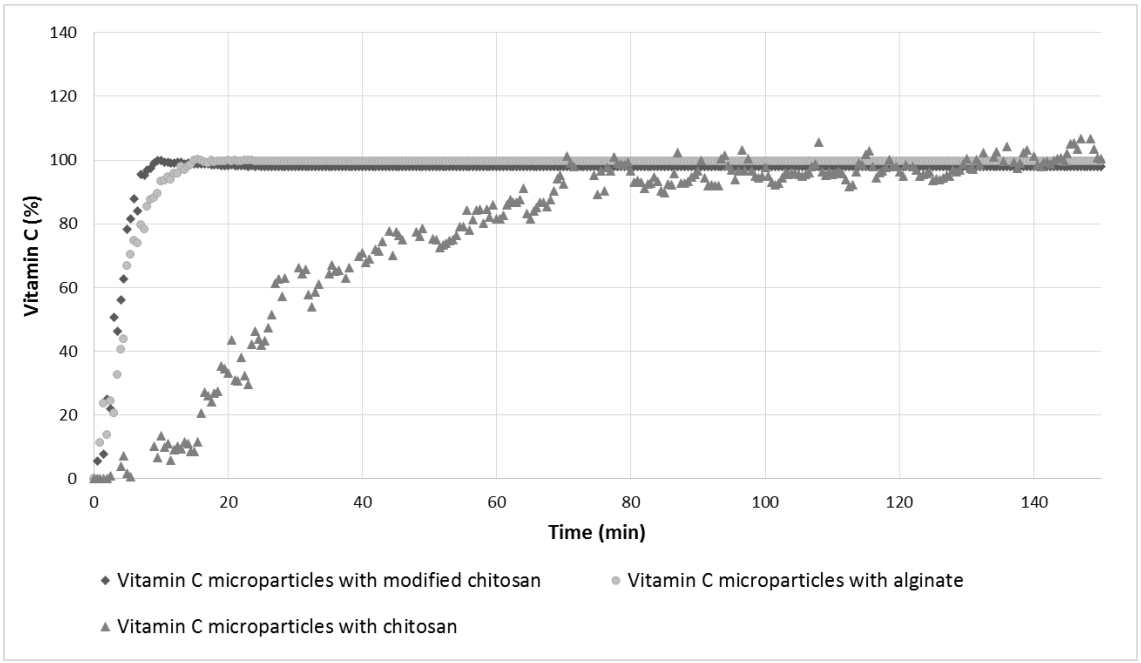

Fig. 5: Release of vitamin C from microparticles made with different encapsulating agents.

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