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RESEARCH ARTICLE

Microencapsulation effectiveness of small active molecules in biopolymer by ultrasonic atomization technique

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Abstract
A method to produce biopolymeric (alginate) microparticles by ultrasonic assisted atomization, previously developed, has been applied to the production of microparticles loaded with a small active molecule (theophylline). Fine loaded alginate droplets have been cross-linked with divalent ions to produce microparticles. Once produced, the particles have been separated by centrifugation or filtration and then they have been dried. Drug release has been evaluated by dissolution tests, dissolving the dried particles in acidic solution at pH 1 for a given time and then at pH 7 to simulate the stomach and intestinal environment, respectively. The encapsulation efficiency and the drug loading have been investigated and the operating conditions have been changed to clarify the role of the transport phenomena on the overall process. To increase the drug loading, shorter separation time and better network's structure were identified as the key operating parameters to allow the process to gain interest from a practical point of view.

Keywords: Biopolymer, theophylline, microencapsulation, ultrasonic atomization

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Introduction

The controlled release of bioactive molecules from polymeric matrices or capsules has been proposed as promising approach in various therapeutic interventions. By microencapsulation techniques, it is possible to protect active molecules and, furthermore, a proper selection of drug-carriers can increase their bioavailability, modifying the release kinetics and improving both drug permanence in blood stream and specific site character. Carbohydrate polymers constitute an ideal class of biocompatible materials[1] to use in microencapsulated pharmaceutical formulations. These polymers offer the possibility to enclose, to protect and to release active molecules in different physiological environment. Their features, as matter of fact, can be regulated by physiological stimuli: inputs such as pH changes, temperature or electric signals, can induce changes in the network structures and, thus, in their functional properties.

In oral administrations, pH-sensitive polymers have an important role in delivery system preparations, because the gastro-intestinal (GI) tract is characterized by a distribution of acidity degree. Among natural polymers, alginates (commercially diffused as salt sodium compound) have a growing interest in enteric pharmaceutical formulations due to their pH sensitive and mucoadhesive features. Alginates are hydrocolloids, water – soluble biopolymers extracted from brown seaweed, their composition consists of alternating blocks of 1-4 linked α-L-guluronic and β-D-mannuronic acid residues. Proportions, distribution, and length of the guluronic and the mannuronic blocks determine physical and chemical properties of the alginate chains including porosity, swelling behavior, stability, biodegradability, and gel strength. At low pH (as the gastric environment), the hydrated sodium alginate is converted in a porous, insoluble skin so encapsulated active molecules are not released. At neutral pH (as the intestinal environment), the alginate skin is converted to a completely soluble viscous layer and, then, the release of previously encapsulated active

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molecules can occur. One of the most important alginate properties is its ability to form gels reacting with divalent cations. Each alginate chain forms junctions with many other chains and as a result gel networks are formed. Due to this ability, alginate microparticles can be produced and used as carriers in the delivery of different kinds of active molecules. The effectiveness of microencapsulation is related to the nature of the drugs (chemistry and size), to the composition of the biopolymer, to the method of production, and to the features of the obtained microparticles (particle's size and network's strength).

Yuk et al.[2] prepared a pH-sensitive drug delivery system which consisted of a core/shell structure using an oil/water (O/W) emulsion technique. The core consisted of oil and dispersed drug (hydrocortisone); the shell consisted of a network which showed a pH-dependant behavior composed by two chemically independent polymers: sodium alginate, which disintegrates in intestinal fluid, and polyacrylic acid, which provides pH-sensitive swelling capacity to the capsule network. These systems had been made dropping the emulsion in a calcium chloride aqueous solution. Beads formation proceeded with the coagulation of aqueous polymer solution mixture used for the capsule network. The emulsion beads showed high pH-sensitive drug release pattern. A detailed investigation of swelling/degradation behavior of ionically cross-linked sodium alginate/chitosan bi-polymeric beads was performed by Bajpai and Tankhiwale[3]. These beads, obtained by dropping the alginate/chitosan solution in reticulating solution, did not have sufficient stability when exposed to environment of varying pH to simulate transition from mouth to colon. The swelling/degradation of various beads was explained on the basis of the electrostatic interactions between alginate and chitosan chains. A method to control the release of vascular endothelial growth factor (VEGF) from small alginate particles, using the dripping technique, was described by Jay and Saltzman[4]. The inability to tune the VEGF release from alginate based systems was overcome mixing population of particles cross-linked by different divalent cations, Zn^{2+} and Ca^{2+}. Ain et al.[5] developed alginate micro-systems as oral sustained delivery carriers for antitubercolar drugs in order to improve patient compliance. The mixture composed of drugs and alginate aqueous solution was dropped through a needle into a $CaCl_2$ solution which was continuously stirred. These droplets formed gel beads instantaneously. Pharmacokinetics and therapeutic effects of antitubercolar drugs encapsulated in alginate microparticles were examined in guinea pigs. Similar procedure was applied by Martins et al.[6] to prepare several types of microspheres composed of alginate, chitosan, and dextran sulphate for studies on oral administration of insulin. By ionotropic gelation, beads were obtained (diameters of the order of millimeters) and characterized. The physico-chemical properties of microspheres composed only of alginate were compared to those of chitosan or dextrane sulphate (or a combination of both polysaccharides) reinforced particles. Dextran sulphate had shown higher protective properties of alginate microspheres under gastric conditions. Furthermore, the addition of polyanionic dextran also increased the encapsulation parameter values. The obtained *in-vitro* release profile revealed that these systems could not only control the protein release. But also were able to decrease the burst release. Wong et al.[7] studied the effects of microwaves treatments on stability and drug release kinetics of alginate, chitosan, alginate/chitosan beads loaded with sulphathiazole. The beads, produced by dripping in different reticulating solutions, were dried by microwaves radiations and subjected to release tests. Microwaves treatments, under specific conditions, had promoted drug release retarding effects. This was explained on the basis of an enhanced crosslinking of the network. Spray coagulation method was found to be suitable for preparation of porous alginate particles. Tu et al.[8] investigated the effect of alginate composition on porosity and the influence of media on the model drug release behavior. Droplets were obtained spraying a sodium alginate solution into a calcium chloride solution through a nozzle at atmospheric pressure. The microparticles were washed, dispersed in water and then frozen using liquid nitrogen; at last they were lyophilized. Mladenovska et al.[9] investigated the influence of polymers type on drug release and potential of chitosan-Ca-alginate microparticles for colon delivery of 5-aminosalycilic acid. These alginate microparticles were prepared using spray drying method followed by ionotropic gelation/polyelectrolyte complexation. Chitosan was localized dominantly on particles wall, while alginate was found inside the particles with a homogeneous distribution. *In-vitro* drug release studies confirmed the potential of these particles to be used for controlled drug release.

Ultrafine particles can be obtained by ultrasound assisted atomization. The main advantage of this technique is the low energy requirement. Moreover, Albertini et al.[10] showed the feasibility of this technique using high viscosity polymer solutions. Rajan and Pandit[11] analysed the impact of physico-chemical properties of liquids (such as flow rate, viscosity, density, and surface tension) and of operating parameters (such as amplitude and frequency of ultrasound, area, and geometry of the vibrating surface) on the droplets size distribution. A correlation was proposed to predict the droplet size formed using an ultrasonic atomizer and it was evaluated against some experimental observation. Small droplets from a polymer (glycerin and carboxy methyl cellulose)–water solutions have been produced by Avvaru et al.[12] with the aim to understand the mechanism by which the ultrasonic vibration at the gas/liquid interface causes the atomization of liquid. Different aqueous solutions having different viscosities and showing both Newtonian and non-Newtonian behavior were used. A correlation has been proposed

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to predict the droplet size based on the dimensionless numbers incorporating the operating parameters of the ultrasonic atomizer and the liquid phase properties. Barba et al.[13] applied ultrasonic atomization process to produce alginate micro-systems. Alginate/water droplets were reticulated using a copper sulfate solution. Several operating parameters (solution concentration, flow rate, atomization power) were changed to study their effects on the produced microparticles size. Literature correlations able to predict the features of the droplets as functions of process parameters were optimized using a statistical approach. Furthermore, the energy requirement for the drops production was compared with the energy required by conventional techniques to evaluate the intensification effect of the ultrasonic on the atomization process.

In this work, starting from the results obtained from a previous work[13], the production of alginate microparticles loaded with a model drug of low molecular mass [theophylline (TP)] by ultrasonic assisted atomization was investigated. In particular, the encapsulation efficiency, the drug loading, and the drug release kinetics have been investigated with the aim to clarify the role of transport phenomena on the overall process.

Materials and methods

Materials

All the materials used were provided by Sigma Aldrich (Milano, Italy). They were: sodium alginate (CAS num. 9005-38-3), calcium chloride (CAS num. 10043-52-4), and TP (CAS num. 58-55-9). Dissolution media were prepared using distilled water, hydrochloric acid, and sodium bicarbonate.

Apparatuses

The used ultrasonic source was the VCX 130 PB (130 W, 20 kHz), the used atomizer tip was the VC 4020 (50 W, 20 kHz). Both these instruments were supplied by Sonics & Materials, Inc., CT, USA). Other laboratory instruments (magnetic stirrer and peristaltic pump) were supplied by Velp Scientifica, IT. The UV-visible measurements were carried out by a UV-visible spectrometer (Lambda 25 by Perkin Elmer).

Methods

Production

Production of particles has been performed preparing an alginate/TP aqueous solution. This solution has been fed into the ultrasonic atomizer by a peristaltic pump. The solution has been atomized for small time intervals (about 10 s) into a beaker containing 80 mL of a stirred calcium chloride solution (concentration 8.9 g/L)[13], in some runs the reticulation medium ($CaCl_2$ solution) also contained the drug. The total amount of atomized solution was about 1.4 mL in each run. When alginate molecules are coordinated by bivalent positive ions, a rigid network forms. The alginate reticulation is a very fast reaction and starts immediately after the impact between alginate/TP drops and calcium solution. Once reticulated, the alginate particles have been separated by centrifugation or by filtration and then they have been dried. A scheme of the protocols adopted along this work is shown in Figure 1.

Dissolution

In order to evaluate the yield of encapsulation, the amount of drug loading, and the release kinetics under physiological conditions, dissolution tests have been performed. Drug release, in conditions simulating the GI tract, has been obtained by dissolving dried particles firstly in a dissolution medium (75 mL, magnetically stirred and kept at room temperature, around 25°C) which was acidic by effect of hydrochloric acid up to pH 1.0 (to simulate the gastric environment) and after 120 min, it was neutralized by adding sodium bicarbonate up to pH 7.0, (to simulate the intestinal environment). The release tests were carried out preliminary using a simplified set-up (beakers kept at room temperature and magnetically stirred instead of the traditional USP-II apparatus) just to evaluate the drug encapsulated and the general patterns of its release

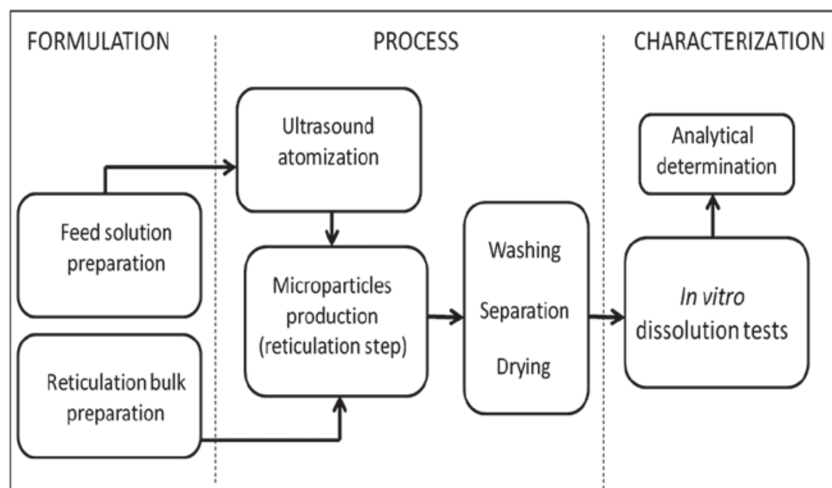

Figure 1. Scheme for the experimental protocols.

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kinetics. During each experiment, at predetermined time intervals, samples of the dissolution medium (2 mL) have been taken and the amount of released drug was evaluated spectroscopically, by collecting the transmission UV spectra in the range 200–400 nm and analysing them as described in the next sub-section. The samples were re-introduced in the dissolution volume after the UV measurement. The released drug (the ratio between the total drug released and the total mass of particles) was evaluated varying the operating parameters, as described in the “Results and discussion” section.

Spectra collection and analysis

The spectra collected during the dissolution tests were used to quantify the amounts of released TP. It is well known that the TP shows an absorption peak around 272 nm, and it is evident also from the example spectrum reported as a continuous curve in Figure 2. Since very low concentrations are expected during the release test, the common approach, based on the simple evaluation of absorbance at a fixed wavelength, was considered here not accurate enough. Indeed, the presence of an unknown quantity of dissolving polymer causes a shift of the spectrum, in the region of interest, which cannot to be predicted. An example of UV spectrum of the polymer at the concentration of 66.4 mg/L is shown in Figure 3. The use of a reference solution containing all the components, but the absorbing one (the drug) is not possible, since the dissolving microcapsules release unknown amount of the polymer together with unknown amount of the drug. The use of a different analytical technique (e.g. an HPLC method) is not possible, either, since the method is destructive and it requires significant amount of sample (2 mL for each measurements, over dissolving volume of about 100 mL). In a previous work, Barba et al.[14] proposed a method based on the fitting of the entire spectrum by a sum of Gaussian curves to separate the influence on the absorption spectra of the TP and of another polymer (Cellulose Acetate Phthalate). A procedure which can be viewed as a simplification of that previously proposed was applied here: in the range between 240 and 300 nm, and an exponential decay function was used as the baseline (the dashed curve in Figure 2) for the TP peak; the results of spectra subtraction (raw data minus the baseline) were fitted by a single Gaussian curve (the dotted curve in Figure 2). The parameter to be related to the drug concentration was thus the height of the fitted Gaussian curve.

Of course, the method was firstly tuned by comparison with a series of standard solutions (prepared with known TP concentration values) in the range of concentrations expected during the dissolution tests (0.5–10 mg/L). The tuning procedure was repeated in both acidic medium (pH 1.0) and in the buffer neutral solution (pH 7.0). The height of the Gaussian was found to be proportional to the drug concentration with a very good correlation (coefficient = 0.0514 L/mg, with $r^2 = 0.9911$ at pH 1.0; coefficient = 0.0535 L/mg, with $r^2 = 0.999994$ at pH = 7.0), confirming the validity of the analytical approach.

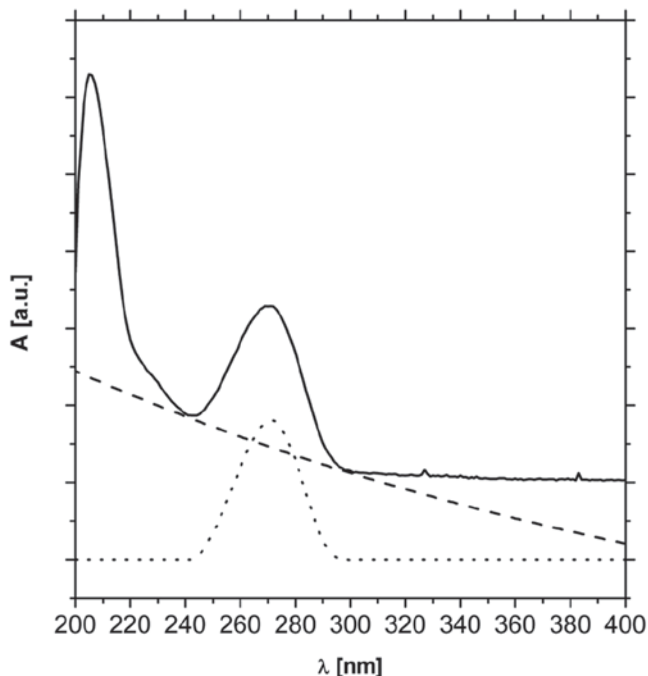

Figure 2. Example of the spectra analysis procedure. Continuous curve, raw data; dashed curve, an exponential baseline; dotted curve, a Gaussian fitted to the spectra subtracted of the baseline, in the wavelength range of interest (240–300 nm).

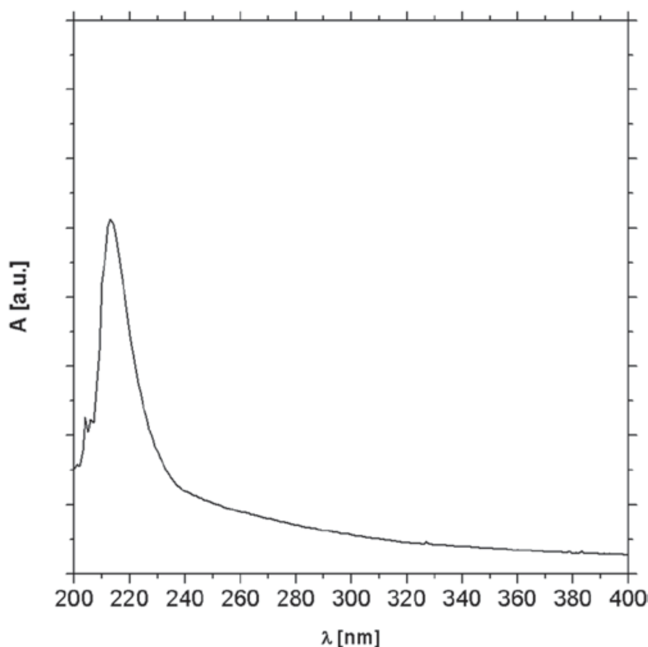

Figure 3. Example of the polymer absorption spectrum at the concentration of 66.4 mg/L.

Results and discussions

As described in the methods section, each experiment involves three steps:

1. The atomization of the solution (alginate or alginate/TP aqueous solution) in the reticulating solution (calcium chloride): During this step, feed composition

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and flow rate, the atomization time, the composition of the reticulating solution and the reticulation time are the operating parameters.

2. The separation and the drying of the microparticles produced. Here, the possible choices are the kind of separation (centrifugation or filtration), the duration and the temperature of the drying process.
3. The release (dissolution) test. In this step, no operating parameter can be varied. For each dissolution run, the absorption spectra were collected every 15 min, both during the acidic dissolution (the first 120 min), and during the following neutral dissolution (the following 60 min). The evolution of the spectra for an example run is reported in Figure 4. The peak around 270 nm (due to the TP absorption) and the shift of the full spectrum from the zero level (due to the alginate dissolution) are both well visible. The spectra are alternatively painted in different gray colors to make the figure more readable. The darker colors identify the spectra collected after the pH raise (after the first 120 min).

Experiments were performed with different TP content in the reticulating solution and separating methods were either filtration or centrifugation. The first experiment, identified as R1 in Table 1, consisted in the atomization of a feed solution (3% alginate, 0.05% TP, giving a theoretical loading ratio of 5/305 = 1.64%) in the reticulating solution, containing only $CaCl_2$; the reticulation was allowed to take place for 5 min before separation. The separation process consisted in three centrifugation steps (at 6000 rpm, during the first step the reticulation solution was removed and then the particle were washed using distilled water). Particles were then dried in an oven at 40°C for 2 h. Due to the small size of the particles (less than 100 μm in diameter) and to their density, which was very close to the water density, the separation was a hard task. The centrifugation steps were performed using the centrifugal filter unit Amicon Ultra (Millipore), based on a small pore cellulose membrane, therefore the separation was very slow.

A sample (of the order of 10 mg) of the obtained powder was soaked into the dissolving medium (75 mL of pH

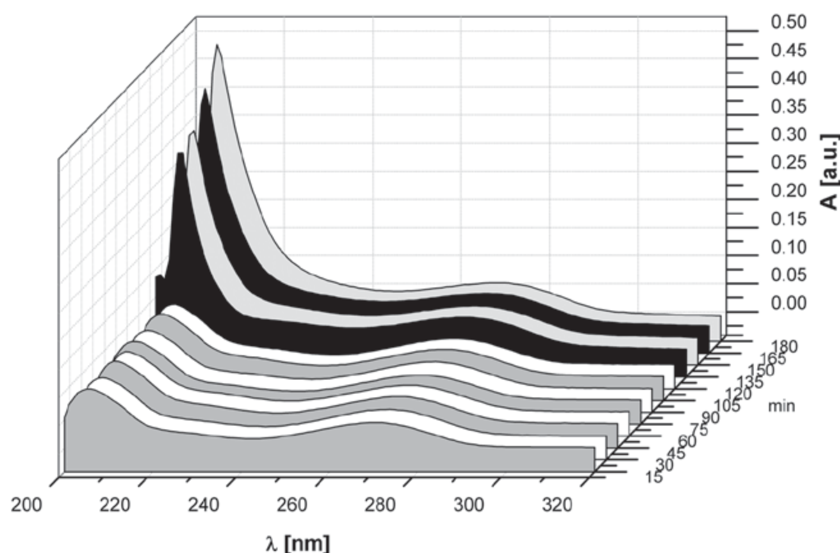

Figure 4. The spectra collected during a release test. Absorbance versus wavelength for several sampling times.

Table 1. Operating conditions (feed composition, atomization parameters, reticulation time, and separation procedure) and the maximum amount of released drug.

Test	Feed	Atomization	Reticulation time	Separation	% TP released
R1	3% Alginate 0.05% TP	$CaCl_2$	5 min	Centrifugation 45 + 30 + 20 min	Negligible
R2	3% Alginate 0.05% TP	Dripped in $CaCl_2$	5 min	Filtration 5 min	1.00%
R3	3% Alginate 0.05% TP	$CaCl_2$ 0.2 g TP	5 min	Centrifugation 45 + 30 + 20 min	0.20%
R4	3% Alginate	$CaCl_2$ 0.2 g TP	5 min	Centrifugation 45 + 30 + 20 min	0.20%
R5	3% Alginate 0.05% TP	$CaCl_2$ 0.2 g TP	30 min	Centrifugation 45 + 30 + 20 min	0.40%
R6	3% Alginate 0.05% TP	$CaCl_2$	30 min	Filtration 5 min	0.13%
R7	3% Alginate 0.05% TP	$CaCl_2$	60 min	Filtration 5 min	0.10%

The theoretical drug ratio is 5/305 = 1.64%.

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1.0 solution) and the dissolution process was performed. The results are reported in Figure 5, the first graph from below, as the released TP versus releasing time. From the visual analysis of the spectra (not reported here), and from the application of the procedure depicted in the previous section, the results are that the released TP was negligible, both in the acidic medium and in the neutral one. A single measurement, after a long time of soaking, was performed (it is not reported in the graph), and it confirmed that no drug was released from the microparticles. Therefore, no drug was loaded in the microparticles during experiment R1.

Such a behavior is not unexpected, since it is well known that low molecular mass drug easily diffuse through alginate gels. Indeed, the pores in the matrix structure were found to be 5–200 nm by electron microscopy, and the cut off size of the pores was found to be 12–16 nm by size exclusion chromatography[15]. Being the TP molecular radius of the order of 0.4 nm[16], it is easy to predict a fast movement of the drug within the polymer network. The estimation of diffusion time could be performed on the basis of diffusivity coefficient of TP in the cross-linked alginate network. Tanaka et al.[17], dealing with glucose diffusing into and out from alginate beads, estimated a diffusivity for the glucose ($C_6H_{12}O_6$, molecular mass = 0.18 kg/mol) in calcium alginate of the order of 6×10^{-10} m^2/s. Grassi et al.[16], dealing with TP ($C_7H_8N_4O_2$, roughly the same molecular mass of the glucose), estimated a diffusivity in sodium alginate membranes of 5×10^{-10} m^2/s. The release kinetics by diffusion from a sphere of radius a is described by the following formula[18]:

$$R(t) = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-n^2 \pi^2 \frac{Dt}{a^2}\right) \qquad (1)$$

The release of the encapsulated drug reaches the 99.9% of its maximum value after 2–3 s, if $D = 5 \times 10^{-10}$ m^2/s and $a = 50$ μm. Therefore, the most probable scenario is: the particles were produced as microspheres made by TP and alginate, but during the reticulation and separation phases, all the drug present in the matrices can diffuse out from the microspheres. Then, during the dissolution test, no further drug remains into the microspheres.

To confirm the key role hypothesized for the diffusion phenomenon on the drug release, larger beads were produced by dripping the alginate/TP solution in the reticulation solution. These beads (3–4 mm in diameter) were quickly filtrated over a paper filter and then they were washed with distilled water. The whole separation process took a few minutes (less than 5 min). The beads were then dried and they were subjected to the dissolution procedure. This test is identified as R2 in Table 1, and its result in term of released TP versus time is reported in the second graph, from below, in Figure 5. In this run, the TP loaded in the microparticles and then released during the dissolution is not negligible, the amount released is roughly the 0.9%, i.e. about the 55% of the theoretical loading (1.64%). It is worth to note that: (1) the release is practically immediate during the dissolution step; (2) practically, the system is non-enteric, since the drug was totally released already during the acidic step. The key role of the diffusion phenomenon on the release process seems to be confirmed. Indeed, the application of eq. (1) to beads with the radius $a = 2$ mm and the same value of diffusivity reported above gives a release of 45% after 3 min (during the separation process). Then, the release process was practically completed within a few minutes (15 min). This is exactly the behavior observed.

Once the key role of the diffusion phenomenon was confirmed, working with large beads obtained by dripping, it should be proved that the same behavior occurs working with particles obtained by ultrasonic assisted atomization. Therefore, a test in which the alginate/TP solution was sprayed into a solution containing not only the reticulating agent ($CaCl_2$), but also the drug to limit the diffusion from the sphere toward the medium. Therefore the test identified as R3 in Table 1 was designed and carried out. In Figure 5, the third graph from below, the release evolution during the test R3 was reported. It is evident, once more, the non-enteric behavior – since all the drug was immediately released already in the acidic stage – but in this case the preparation process causes

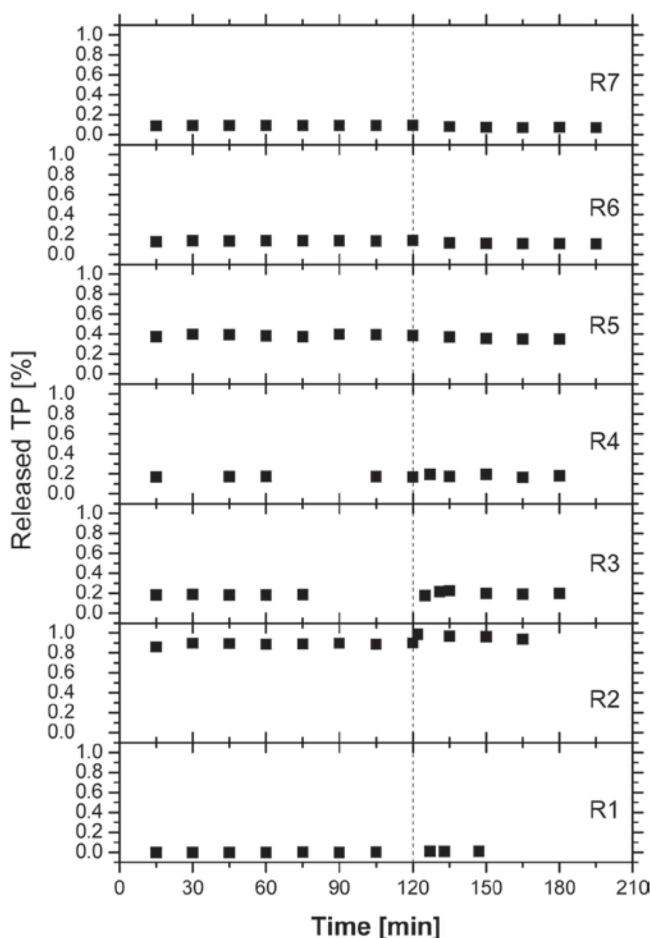

Figure 5. Percentage of released drug versus time for each one of the runs listed in Table 1.

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some drug to be entrapped in the microspheres (0.20%, over the theoretical value of 1.64%).

To be sure on the role of the diffusion in the process, one last test was designed and carried out, identified as R4 in Table 1. In this run, the atomizing solution was made using only the alginate solution, carrying no drug, and the reticulating solution contained both the reticulation agent ($CaCl_2$) and some drug. By this way, if the controlling phenomenon is the diffusion, the microspheres should absorb the drug from the reticulation medium. The release evolution, reported in Figure 5, shows the non-enteric behavior already observed in previous tests (i.e. all the drug loaded was immediately released, already during the acidic step). The asymptotic value of the released drug, which should be equal to the drug loaded during the reticulation step, was in this case roughly 0.2%, i.e. the same value obtained during test R3. This result confirms the main role played by the diffusion (in this case, the drug has diffused into the beads, coming from the outer medium), and also states that the value observed (0.2%) has to be intended as the solid phase concentration which is in equilibrium with the reticulating solution concentration (2 g/L).

With the aim of increasing the drug loading, the reticulation step was extended up to 30 min in test identified as R5 in Table 1, the reticulation time being the single parameter changed with respect to test R3. Indeed, a better reticulation should decrease the diffusion coefficient and/or to increase the equilibrium value because of a more structured gel network. As expected, it could be seen, from the fifth graph from below in Figure 5, that the release evolution has a higher asymptotic value, the drug loading being the 0.4%. Once more, the system behaves as a non-enteric one (the drug was totally released already during the acidic step).

Once clarified the role of the transport phenomena in the production process and the advantage of a larger reticulation time on the drug loading, with the aim of pointing out a potentially useful process, some improvement should be made. In particular, a reticulating solution rich in the drug is of poor practical interest (both for separation problems and for the cost of the drug), and also the separation process is a major drawback, because of its complexity and of its length in time. Therefore, another test was designed and carried out. The separation process was replaced by a filtration step instead of the centrifugation, and the drug was removed from the reticulating solution (test identified as R6 in Table 1). The drug release evolution is reported in Figure 5 (the sixth graph from below), it could be noticed that: (1) the release pattern is coherent with a non-enteric behavior, (2) the asymptotical value is reached immediately (the system is an immediate release one). The drug loading is very low, just above 0.13%. A comparison between the test R1 (the seminal experiment) and the test R6 gives the result of a low but not negligible drug loading, because of a longer reticulation time (30 min versus 5 min) which gives a more structured gel network, and because of a faster separation step (5 min versus 90 min), which decreases the time allowed to the drug to diffuse out from the microspheres.

With the aim of a better reticulation, one more test was performed, identified as R7 in Table 1. In this case, the reticulation time was raised up to 60 min. The release evolution is reported in the graph on the top in Figure 5. The usual non-enteric behavior was observed, and the drug loading results to be very low, even lower than the result obtained in test R6. Probably the longer reticulation time did not improve the structure of the gel network, but the longer time in the reticulation solution allowed the release of the drug by diffusion.

The dissolution tests were performed at 25°C instead of the physiological temperature. Because of the very low level of entrapped drug, in each run tested, there was no interest in testing the microparticles in the more traditional way (at 37°C in USP-II apparatus). The increase in the release medium temperature, furthermore, would increase the drug diffusivity and thus the release kinetics (the scarce drug loaded will be released even faster than at 25°C).

The following experimental observations permit to define the conditions which would enhance the effectiveness of ultrasound assisted microencapsulation of low molecular mass drug:

1. Larger particles size (obtainable by varying the process parameters such as the ultrasound frequency, or the polymer solution concentration) causes a slower release and thus enable higher drug loading;
2. Lower separation time (filtration instead of centrifugation) avoids drug losses in the washing media;
3. Better network's structure could give a tailored release profile. The network's strength could be improved by increasing the reticulation time and/or the reticulating agent concentration. Furthermore, new protocols where physical (e.g. microwaves curing) or reactive chemical (aldehydes) agents could be used to enhance the network crosslinking to improve encapsulation efficiency and to avoid undesirable drug losses.

Conclusions

In this work, the production by ultrasonic assisted atomization of alginate microparticles loaded with a drug of low molecular mass (TP) was investigated; the effect of some of the operating parameters was investigated.

The efficiency of the drug encapsulation in the polymeric beads was low, and the key role of the transport phenomena (diffusion) was confirmed by varying the particle size, by adding the drug also in the reticulating solution, by varying the reticulation/separation time. Indeed, the loading ratio was found to increase: (1) producing large particles (the drug diffusion takes place over a time interval larger than the reticulating time); (2) carrying out the reticulation in a bulk containing the drug,

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the drug diffusion from the particle decreases by effect of the drug in solution; (3) carrying out the reticulation and the separation steps quickly (since the drug diffusion is shortened). However, a positive effect on the drug loading (an increase) was observed as consequence of a prolonged reticulation. Furthermore, under the operative conditions investigated along this work, the enteric behavior was never observed, since the release profiles are not different during the dissolution at two different pH levels.

The main results of this work, therefore, are:

1. The better knowledge of which phenomena are relevant to the encapsulation of low molecular mass drugs in alginate beads;
2. The proof of feasibility for the encapsulation of drug by ultrasonic assisted atomization; and
3. The microparticles made of TP/Alginate reticulated with calcium ions were not suitable to produce enteric pharmaceutical forms.

Declaration of interest

The authors report no conflicts of interest.

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