Controlled Release of Drugs from Microparticles Produced by Ultrasonic Assisted Atomization Based on Biocompatible Polymers

A. A. Barba,[a,*] A. Dalmoro,[a,b] M. d'Amore,[a] and G. Lamberti[b]

[a]Dipartimento di Scienze Farmaceutiche e Biomediche, Università di Salerno, Via Ponte don Melillo, I-84084 Fisciano (SA)

[b]Dipartimento di Ingegneria Industriale, Università di Salerno, Via Ponte don Melillo, I-84084 Fisciano (SA)

Original scientific paper
Received: May 5, 2012
Accepted: July 25, 2012

Microencapsulation of active molecules in biocompatible polymers is a matter of great interest in pharmaceutical sciences. Ultrasonic assisted atomization as a new technique to produce microencapsulated systems seems to offer several advantages (low level of mechanical stress in materials, reduced energy request, reduced apparatuses size) with respect to more conventional techniques.

In this work, fine drug-loaded particles were produced by ionic reticulation of droplets obtained by ultrasonic atomization of biopolymers solutions. The particles were then characterized in terms of morphology and drug release kinetics. Data were used to estimate the PNMS (Polymeric Network Mesh-Size) with the aims of clarifying its role in controlled drug release, and analyzing its relationships with material and process parameters.

For materials and operative conditions investigated, the calculated PNMS was found consistent with a fast release of drugs of small molecular size.

Key words:
Microencapsulation, ultrasonic atomization, Polymeric Network Mesh-Size (PNMS)

Introduction

Current approaches in the production of drug delivery systems are focused on two main goals: to enhance manufacturing processes in response to the need for reducing energy consumption, emissions, wastes and risks; to innovate the functionality of new formulations using biocompatible materials[1,2,3]. Microencapsulation of active molecules is the manufacturing technique that seems to better respond to the requirements above as is shown by the continuous development of new technologies, both in academic and industrial fields. In 2002, over 1000 patents were filed concerning various microencapsulation processes[4]. Relevance of encapsulation techniques consists in the effectiveness to protect many kinds of active molecules (drugs, gene, anticancer chemical drugs, vitamins and proteins) through their envelopment in suitable carriers. By this way, the overcoming of serious problems associated to poor stability in physiological environments and low absorption of many drugs and to their controlled release is achieved.

Microencapsulation processes can be performed by both physical-chemical and mechanical processes[5,6,3]; all of them sharing the following fundamental steps: incorporation of bioactive compounds; droplets formation; solvent removal; microparticles harvest and stabilizing treatment[7,8]. The suitable technique is selected on the bases of the polymer nature, the desired particles size and the chemical features of drug, with a special concern to the drug solubility in the polymeric material[9].

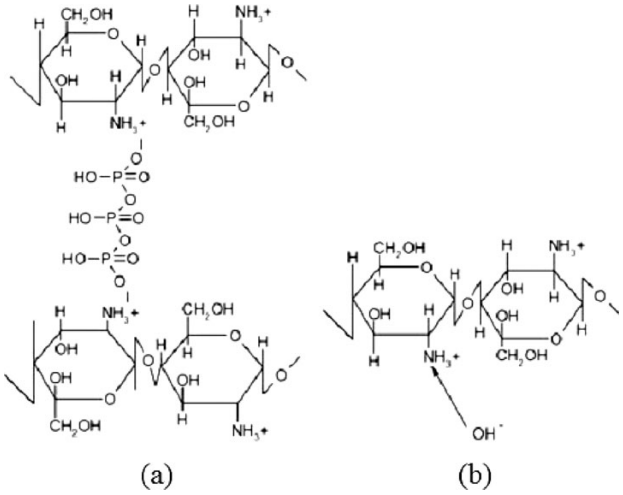
Among the mechanical processes, microencapsulation by ultrasonic assisted atomization is characterized by several interesting features. Briefly, small droplets of a uniform size may be formed by feeding a fluid, at a controlled rate, through a small orifice in the tip of a horn vibrating ultrasonically in a longitudinal mode[10] (ultrasonic frequency allowed: 18–100 kHz). Vibration energy causes formation of liquid threads and then of droplets. Many literature references report studies that explain the liquid disintegration by ultrasonic mechanism[11,12]; cavitation and capillary wave mechanisms theories are the most accredited[13]. Recent popularity of the ultrasonic atomization can be mainly attributed to its ability to produce drops of small size and low inertia since it works involving low velocities. This feature allows reducing the energy request respect to the more conventional atomization systems (nozzle atomizers, two fluid atomizers), the volume of apparatuses (compact equip-

*Corresponding author: Anna Angela Barba; Tel: +39-089969240; Fax: +39-089969602; E-mail address: aabarba@unisa.it

ment) and the mechanical stress of feed avoiding deactivation of bioactive substances.

In our previous works[14,15] alginate polymers were tested to produce microencapsulated pharmaceutical formulations by ultrasonic atomization. The impact of physico-chemical properties of liquids (flow rate, viscosity, density and surface tension) and of operating parameters (amplitude and power of ultrasound) on the droplets size distribution and the role of transport phenomena in drug release kinetics were analyzed. In this work, chitosan is selected to encapsulate a model active molecule because it shows, as many carbohydrate polymers, interesting features as biocompatible carrier for drug release systems production.

Chitosan is a natural cationic polysaccharide composed of glucosamine and N-acetyl-glucosamine residues derived from partial deacetylation (degree deacetylation, DD, performance) of chitin, which is generally obtained from crustacean shells (Fig. 1). Chitosan, being a cationic polysaccharide in neutral or basic pH conditions, contains free amino groups and is hence insoluble in water. In acidic pH, amino groups can undergo protonation, thus making chitosan soluble in water. Usually 1–3% aqueous acetic acid solutions are used to solubilize it[16,17]. Chitosan presents features that make it useful for preparation of drug encapsulating microparticles. These include: ability to control the release of active agents; possibility of avoiding the use of organic solvents since it is soluble in aqueous acidic solution; easiness of cross-linking, being a linear polyamine containing a number of free amine groups that are readily available for this purpose; increased residence time at the site of absorption due to its mucoadhesive character[16].



F i g . 1 – *Chitosan structure and prevalent kinds of reticulation: by tripolyphosfate ionic crosslinking (a, at low pH); by deprotonation (b, at high pH)*[26]

Chitosan-based particulate systems are designed for pharmaceutical and biomedical applications. Some of them are:

– colon targeted drug delivery, since chitosan can be biodegraded by colonic bacterial flora and has mucoadhesive character (to prevent drug loss at stomach and small intestine, thereby facilitating quantitative drug delivery to the colon, chitosan microparticles have to be coated by enteric materials, such as Eudragit)[18];

– bioadhesive dosage forms for mucosal administration (ocular, nasal, buccal, gastro-enteric and vaginal-uterine therapy);

– gene therapy. Chitosan could ionically interact with the negatively charged DNA and forms polyelectrolyte complexes, where DNA becomes better protected against nuclease degradation, leading to better transfection efficiencies[16,19];

– drug targeting to tumors. In fact, tumor tissues exhibit lower pH (varying from 5.7 to 7.8 depending on tumor histology and volume) than pH of body tissue and of blood, which are around 7.4[18].

Detailed reviews of new developments on chitosan-based bioapplications are reported in Agnihotri et al.,[16] and Dash et al.,[20]. On the microencapsulation of active molecules in chitosan polymer by ultrasonic assisted atomization several researches are reported in Albertini et al.[21], Klaypradit et al.[22], Pamujula et al.[23]

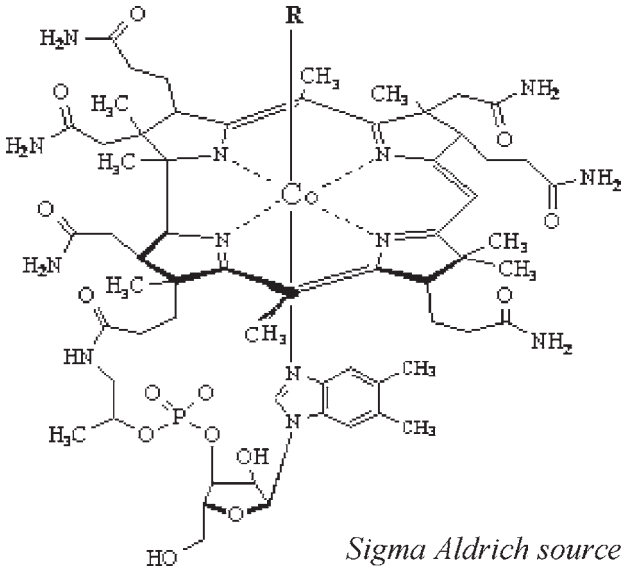
Aims of this work were the production of fine drug-loaded particles produced by ionic reticulation of droplets obtained by ultrasonic atomization and the study of their drug release properties. In particular, the data are used to estimate the PNMS (Polymeric Network Mesh-Size) to clarify its role in controlled drug release, and the relationships holding between material and process parameters and PNMS itself.

Experimental

Materials

Chitosan (CS) (CAS no. 9012-76-4, medium molecular weight, DD 75–85%) and B12 vitamin (B12) (Sigma Aldrich, CAS no. 68–19–9) (Fig. 2) were purchased from Sigma Aldrich s.r.l., Milano IT and were used without further purification treatments.

Acetic acid (AcA) (CAS no. 64-19-7) was used to prepare water solution of chitosan (acidifying agent); sodium tripolyphosphate (TPP) (CAS no. 7758-29-4, purity ≥ 98%) and hydrochloric acid (CAS no. 64-19-7) were used in water solutions as cross-linking and acidifying agents, respectively; hydrochloric acid, sodium hydroxide (CAS no.



Alternative name: cyanocobalamin
Molecular formula: C63H88CoN14O14P
Molecular weight: 1355.37 uma
Commercial form: dark red crystalline powder
Stokes radius (r_{Stokes}): 0.86 nm
Water solubility: high
Diffusivity (in water at 37°C): $3.8 \cdot 10^{-10}$ m^2/s

Fig. 2 – *B12 structure and its some properties*

1310-73-2) and potassium dihydrogen phosphate (CAS no. 7778-77-0) were used as buffer solution components. All the listed products were purchased from Sigma Aldrich s.r.l., Milano IT.

Methods

Chitosan/B12 solution preparation. Several batches of CS/B12/water solutions were prepared by adding first B12 powders, 0.2 % w/v, in acidulate distilled water (by 1% v/v of AcA), then after a gently mixing, 1% w/v of CS powders were added. The preparing method was performed at room condition; mixing was prolonged until obtaining a homogeneous solution.

Crosslinking solution preparation. TPP powders, 5 % p/v, were dissolved in distilled water to obtain a solution with initial pH of 9 ca. Since better reticulation conditions are achieved at low pH, drops of hydrochloric acid were added to reach pH 3.

Chitosan/B12 particles production and characterization. Chitosan ionic gelation, i.e the complexation between oppositely charged macromolecules to give a network structure (reticulating process), is the method that has attracted the greatest attention in the gel-sphere preparation for the mild-condition of the process and for its simplicity[16,24]. Furthermore, the ionic gelation method is characterized by a reversible physical crosslinking due to electrostatic interaction instead of a chemical crosslinking, and thus it can be applied avoiding possible toxicity of reagents. Briefly, in this study, the ionic gelation method was applied as follows: chitosan was dissolved in aqueous acidic solution to obtain positive charges on polymeric chains (NH_3^+ groups by amine protonation). This solution was then added within the tripolyphosphate solution (where $–P_3O_{10}5–$ groups were contained). Due to the complexation between oppositely charged species, chitosan undergoes ionic gelation and precipitates to form spherical particles. It is worth to note that the ionic gelation performance is affected from the degree of deacetylation (DD) and the molecular weight (Mw) of the chitosan . High percentage of DD allows increases of free amino groups, consequently more strong crosslinkings are obtainable even if, in general, TPP/CS microparticles have intrinsically poor in mechanical strength that is one of the limits in their usage in drug delivery[16].

In this work, two kinds of particles, both considered as matrix structures, were produced using the home-made apparatus sketched in Fig. 3. It consists by a VCX 130 PB ultrasonic processor (130 W, 20 kHz, Sonics & Materials Inc., CT, USA) and a

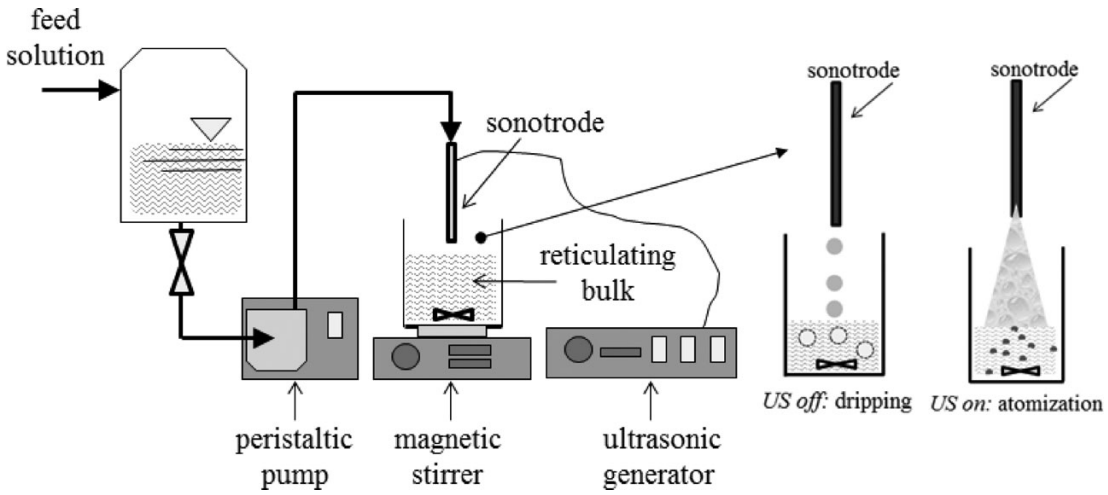


Fig. 3 – *Sketch of the home-made apparatus assembled and used to produce chitosan/B12 particles*

standard tip (sonotrode, i.e. a steel channel with inner diameter of $2.3 \cdot 10^{-3}$ m) for low atomization rate (up to 1 mm^3/s), code VC 4020 (50 W, 20 kHz, Sonics & Materials Inc., CT, USA) as sources of ultrasonic vibration and atomizer, respectively. Feed solution is delivered to the sonotrode by a peristaltic pump (Velp Scientifica Italia, Usmate, MI, ITALY). The produced drops are collected in the reticulating bulk placed on a magnetic stirrer. The distance between the atomizer tip and the reticulating solution is maintained at approximately 2 cm.

In particular, several batches of millimetric particles were obtained by dripping method, i.e. keeping the ultrasonic generator off (Fig. 3, *US off*). By this way was reproduced the most common technique used to produce beads in reticulating bulk. Microparticles of CS/B12 were produced activating the ultrasonic generation (Fig. 3, *US on*). In Table 1 the main operative parameters were summarized.

Both kinds of particles were obtained by crosslinking phenomena between chitosan polymeric chains and tripolyphosphate groups. After a given time of exposure to reticulation, rubbery particles were obtained. Then, they were separated by a filtration process, washed by phosphate buffer solution, photographed (by Canon digital camera, IXUS 850 IS, by Leica digital camera DFC 280 mounted on optical microscope Leica Microsystems DM-LP, Wetzlar, GERMANY) and subjected to diameter measurements by image analysis (using the public domain software ImageJ 1.40g, Wayne Rasband, National Institutes of Health, USA, freely available at http://rsb.info.nih.gov/ij/). Finally, produced particles were stabilized by convective drying in oven at 50°C until reaching constant weight. Moisture content of fresh and dried particles was assayed by a moisture balance (OHAUS mod MB45, OHAUS Europe, Nänikon, SWISS).

All kinds of produced particles were subjected to dissolution tests to evaluate the yield of encapsulation, the amount of drug loading, and the release kinetics under physiological conditions. In particular, the dissolution tests were carried out putting given amounts of particles in a beaker where stirred buffer solution at pH 7.4 was kept at 37°C. B12 in dissolution bulks was assayed by spectrophotometric analysis (Lambda 25 UV/VIS Spectrometer, PerkinElmer, Waltham, MA, USA) at 361 nm. Since particles were produced in a low amount per test, the conventional USP Apparatus 2 cannot be

Table 1 – *Selected operative parameters*

Droplet production / Operative parameters	By dripping technique	By ultrasonic assisted atomizazion
	Droplets production	
Chitosan concentration	1% w/v	1% w/v
B12 load	0.2 % w/v	0.2 % w/v
Feed solution rate	4.8 mL/min	4.8 mL/min
Ultrasonic amplitude	--	98%
	Particles production	
Tripolyphosfate solution conc.	1% w/v	1% w/v
Volume reticulation solution	100 ml	100 ml
Reticulating solution pH	3	3
Stirring condition	150 rpm	150 rpm
Reticulation time	3 min	3 min
Separation technique: filtration	yes	yes
Washing medium (phosphate buffer pH 7.4)	yes	yes
Particles characterization	diameter, encapsulation efficiency, drug load, release properties	diameter, encapsulation efficiency, drug load, release properties
	Particles stabilization	
Convective drying (in oven at 50°C)	yes	yes
Particles characterization	diameter, volumetric shrinkage, release properties	diameter, volumetric shrinkage, release properties

used because it requires a large volume of dissolution and then diluted solution for which UV–vis spectrophotometry method was proved to be inaccurate to assay the B12 releases. All the experimental tests were performed at minimum in triplicate.

Results and discussion

Process parameters setting. The production of B12 loaded microparticles by ultrasonic atomization was preceded by several preliminary activities focused on the setting of the suitable operative parameters to be adopted. Since many factors affect both drug entrapment efficiency and drug release properties (process parameters and materials properties), polymer concentration, feed solution rate, concentration, pH and bulk quantity crosslinking solution, reticulation time and stirring velocity were investigated. To better observe the influence of the above listed parameters millimetric particles were produces using the apparatus showed in Fig. 3. with the ultrasonic generator off (dripping method).

Generally, a low concentration of chitosan corresponds to a low encapsulation efficiency and a poor particles consistence; however, at higher concentrations, chitosan forms highly viscous solutions, which are difficult to process[17,25]. For this reason an optimum of 1% w/v of chitosan concentration was selected. Feed solution rate was chosen to guarantee a full and stable atomization at conditions of ultrasonic generator on (selecting also the amplitude percentage of the ultrasonic signal). Crosslinking solution concentration has an effect on particle strength even if its effect must be discussed together with the solution pH values. At low pH (typically 3), phosphoric ions are predominantly involved in the crosslinking. At high pH (typically 9) both the OH^- and phosphoric ions are present, and compete with each other to interact with the $-NH_3^+$ sites of chitosan (OH^- ions are linked to the amino group by deprotonation, see sketch in Fig. 1). Starting from swelling and elemental measurements, and SEM investigation Bhumkar et al.,[26] found that chitosan crosslinked structure obtained at pH 3 had a higher density (and lower porosity) then those reticulated at pH 9. On the bases on literature studies, in this work to obtain particles with more consistence (to allow an easy separation step), the reticulation solution was acidified by hydrochloric acid until pH 3. The reticulation volume was fixed to 100 mL to guarantee an opportune bulk stirring velocity. This latter parameter was optimized to efficiently disturb the surface of the liquid bulk so as to avoid any accumulation of drops on liquid surface that could obstruct the droplets entrance into the reticulating bulk. However, high stirring velocity had effect on morphology as this could lead to prolate or disrupted particles. Several tests to define the opportune crosslinking time were, at last, carried out. Prolonged times were revealed to be non-significant to obtain an increase in consistence particles. They rather allowed high losses of loaded active molecule in the crosslinking bulk. Thus, 3 minutes were selected as the overall reticulation time: i.e. the time was counted from the start of dripping or atomization processes, extended at 2 min, and after 1 more minute at feed solution switched off. In Table 1 the selected operative parameters were summarized.

Particles features and release properties. In Fig. 4 photos of both fresh millimetric and microparticles were shown. In Table 2 main properties of both kinds of particles were reported. Millimetric (mean diameter: 5.40 mm ± 0.40) and microparticles (mean diameter: 790 μm ± 0.38) were characterized by spherical morphologies and polymeric-shell liquid-core structures. Diameters and shell thickness were observed and measured by imagine analysis of digital photos.

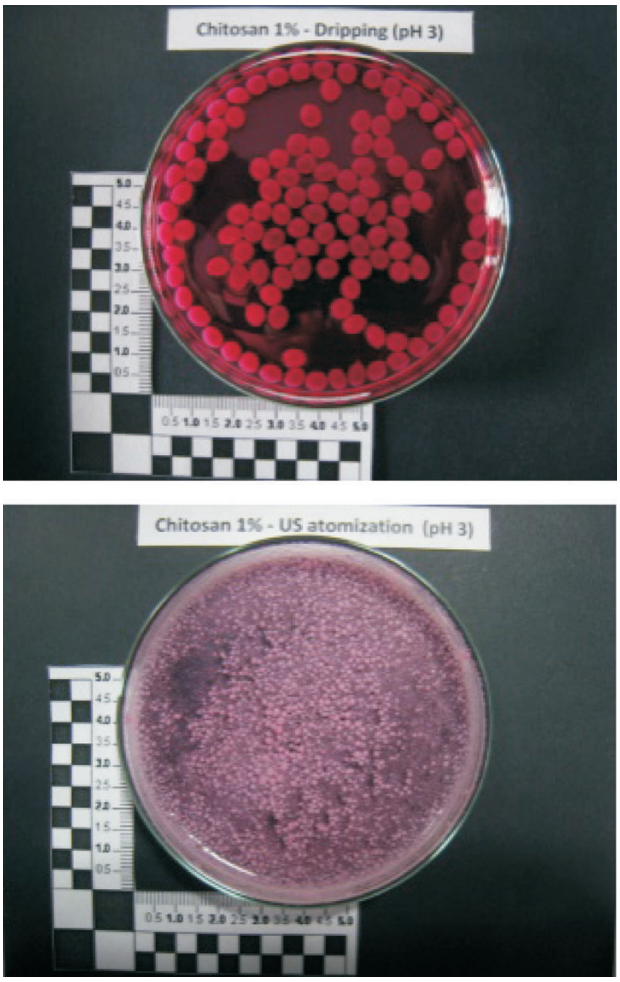


Fig. 4 – *Fresh millimetric particles and microparticles of chitosan/B12*

Table 2 – *Main properties of fresh and dried particles obtained by dripping and ultrasonic atomization processes.*

	By dripping technique	By ultrasonic assisted atomizazion
Fresh particles:		
mean diameter	5.40 mm ± 0.40	790 μm ± 0.38
moisture content	95%	92%
drug load	3.7%	< 1%
encapsulation efficiency	66%	12%
Dried particles:		
mean diameter	3.75 mm ± 0.58	613 μm ± 0.23
volumetric shrinkage	66 %	53 %
residual moisture content	0.76%	1.76%

Encapsulation efficiency (EE) of both kinds of loaded particles was assayed by destructive dissolution tests performed putting given amounts of fresh washed particles in known volumes of buffer solution. Entrapped B12 (mg) was then measured by spectrophotometric method. The theoretical B12 mass was calculated starting from the values of B12 concentration in feed solution and the used feed solution volume both in dripping and atomization processes. Eq. 1 was applied to calculate the encapsulation efficiency:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{B12 mass assayed by dissolution test}}{\text{B12 theoretical mass}} \cdot 100 \quad (1)$$

In millimetric particles the EE assayed was of 66% (± 5.1) whereas in microparticles 12% (± 0.53) was determined. These different performances were due to the different size (see different surface areas) of particle exposed to reticulation bulk where large amounts of B12, especially in microparticles batches, were measured.

Drug content (DC) of both kinds of loaded particles was also assayed (starting from the ratio between values of the effective B12 encapsulated mass and the dried total mass per 100) achieving 3.7 % (± 0.21) and 0.91 % (± 0.17) for millimetric- and micro- particles, respectively.

In Fig. 5 the B12 release profiles from fresh chitosan particles were reported. They were shown a very fast B12 release rate: for both the two kinds of particles after 30 minutes more than of 70% of active molecule was transferred in the dissolution bulk. Moreover erosion phenomena were not observed. All this, together to the high losses of B12 in the reticulating bulk (or otherwise the low EEs achieved) suggested that diffusion was the

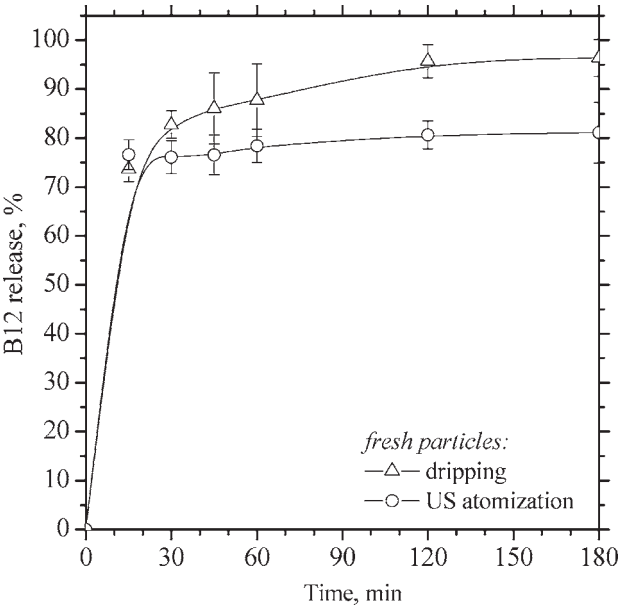


Fig. 5 – *B12 release profiles from fresh chitosan particles (the error bars refer to the standard deviation SD)*

dominant transport phenomenon through the shell of particles. Despite relative large size and molecular weight of B12 molecule (r_{Stokes} = 0.86 nm, Mw ~ 1400 uma) it was appeared reasonable to extent to chitosan reticulated structure some hypotheses and study approaches developed on the diffusion of small molecules in alginate crosslinked gel[15,2]. In particular, polymeric network mesh-size (PNMS) was the first imputed factor placed under investigation.

PNMS estimation. To evaluate the release kinetics and the influence of the shell-core structure of chitosan particles on transport phenomena, a simple model of the drug release has been proposed. A number N of small particles of external radius a (and thus of volume $V_1 = (4/3)\pi a^3$) were immersed in a volume V2 of dissolution medium, initially drug-free, $C_A^0(t = 0) = 0$, stirred and thermostated. The particles have a shell of thickness δ, and are practically all of the same size (mono-disperse distribution is usually achieved both by dripping and by ultrasonic assisted atomization). The situation is depicted in Fig. 6. In the inset, size and concentration of the drug (component "A") in the core, C_A^I, in the shell, C_A^P, and in the dissolution medium, C_A^0, are reported. The resistance to the transport is hypothesized to be localized only in the shell, therefore the mass flow rate of the drug from a single particle is:

$$W_A = (4\pi a^2)\frac{D}{\delta}(C_A^I - C_A^0) \quad (2)$$

The two mass (drug) balances in the N particles and in the dissolution medium V_2 are:

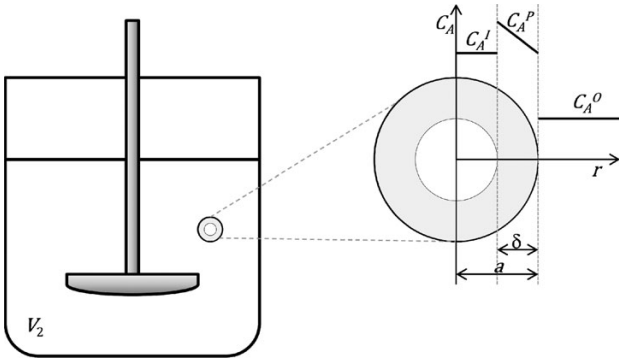


Fig. 6 – *Schematic of the drug release tests and of the main hypotheses of the model-based analysis*

$$\begin{cases} NV_1 \dfrac{\mathrm{d}C_A^I}{\mathrm{d}t} = -NW_A & \text{I.C.}\ C_A^I(t=0) = C_{A0}^I \\ V_2 \dfrac{\mathrm{d}C_A^0}{\mathrm{d}t} = NW_A & \text{I.C.}\ C_A^I(t=0) = 0 \end{cases} \quad (3)$$

The two ODEs (3) can be analytically solved, making also use of eq. (2). The evolution of the concentration in the outer phase (the dissolution medium) will be:

$$C_A^0(t) = N \frac{4\pi a^2}{V_2} \frac{D}{\delta} C_{A0}^I \tau \left[1 - \exp\left(-\frac{t}{\tau}\right)\right] \quad (4)$$

In eq. (4) the time constant τ is:

$$\tau = \frac{1}{4\pi a^2 \dfrac{D}{\delta}\left(\dfrac{1}{NV_1} + \dfrac{1}{V_2}\right)} \quad (5)$$

An estimate of the initial drug loading concentration, C_{A0}^I, could be easily obtained knowing that the initial drug ($C_{A0}^I NV_1$), at the steady state, will be present in the dissolution medium (concentration equal to C_{ASS}^0, mass equal to $C_{ASS}^0 V_2$) in equilibrium with some drug still confined into the microparticles themselves ($C_{ASS}^0 NV_1$, the concentration in the outer phase, C_{ASS}^0, being the same of the concentration inside the microparticles, C_{ASS}^I). Therefore, the initial drug loading concentration is:

$$C_{A0}^I = C_{ASS}^0 \left[1 + \frac{V_2}{NV_1}\right] \quad (6)$$

The raw release data, i.e. concentration of the B12 in the dissolution medium from which data in Fig. 5 have been calculated, can be taken as the basis for the depicted analysis. Once all the parameters (a, N, d, C_{ASS}^0) are known or estimated (see Table 3), the fitting of eq. (4) to the experimental data allows to calculate the diffusivity of B12 through the polymeric shell (D, which is the only unknown in eq. (4) once the evolution of B12 concentration in the dissolution medium is known). Table 3 reports the values calculated, by this way, for the diffusivity in the two systems: $D = 2.18 \cdot 10^{-10}$ m^2/s for particles obtained by dripping and $D = 1.06 \cdot 10^{-10}$ m^2/s for particles obtained by ultrasound assisted atomization. The two techniques, beyond to give particles of different size, could produce different microstructures, either because of the effects of the ultrasound on the gel network, and because of the kinetics (and the extent) of the reticulation which is affected by the size of the produced droplets.

The diffusivity of B12 in water is known to be $D_0 = 3.80 \cdot 10^{-10}$ m^2/s, and the Stokes (hydrodynamic) radius of the molecule is $r_{Stokes} = 0.86$ nm. The ratio D/D_0 for the two systems therefore assumes the values $D_{dripping}/D_0 = 0.574$ and $D_{atomization}/D_0 = 0.278$. Lustig and Peppas [27] proposed, and Amsden [28] extensively tested, a relationship obtained starting from the volume free theory:

$$\frac{D}{D_0} = \left(1 - \frac{r_s}{\xi}\right) e^{\left(-Y\frac{\varphi}{1-\varphi}\right)} \quad (7)$$

In which r_s is the Stokes radius, φ is the polymer volume fraction ≈ polymer mass fraction ≈ solid fraction = 0.07 (particles mean moisture: 0.93) of particles, and ξ is the network mesh size. According to Lustig and Peppas, the coefficient Y can be taken as unity, and this allow to calculate radius mesh size of $\xi_{dripping} = 2.3$ nm and of $\xi_{atomization} = 1.2$ nm. Both values resulted larger than the B12 Stokes radius.

Drying process and its effects. As reported in section Experimental, fresh chitosan particles were stabilized by drying convective treatments. The main characteristics of the dried systems achieved were shown in Table 2. Millimetric particles were subjected to a greater volumetric shrinkage than the microparticles (66 % vs 53 %); on the contrary, the

Table 3 – *Parameters and results of the model-based analysis of the drug release kinetics data*

	a mm	N …	δ μm	C_{ASS}^0 mg/L	D m^2/s	D/D_0 …	ξ nm
dripping	2.700	50	250	119.2	$2.18 \cdot 10^{-10}$	0.574	2.3
ultrasonic assisted atomization	0.395	5300	10	40.0	$1.06 \cdot 10^{-10}$	0.278	1.2

residual moisture contents determined was higher in microparticles than in millimetric particles. As hypothesized in the Mesh size estimation paragraph, a different structural arrangement occurred in the particles formation under the different process conditions, so the same water equilibrium contents could be not achieved.

In Fig. 7 the B12 release profiles from dried chitosan particles were reported. As can be seen, drying process did substantially not affect the release kinetics. Indeed, fast B12 diffusion was observed for both kinks of dried particles (the 80% of B12 load was released in about 30 minutes). In particular, due to the larger exchange area, loaded microparticles exhibited a B12 percentage releases over 70% within the first 10 minutes. With respect to the B12 release profiles assayed starting from fresh particles, a little delay in the B12 diffusion was observed. It was attributed to the time required to wet and plasticize the chitosan reticulated chains. Thus, the applied drying conditions, although leading a volumetric shrinkage by more than 50%, globally did not affect the release properties of chitosan/B12 particles.

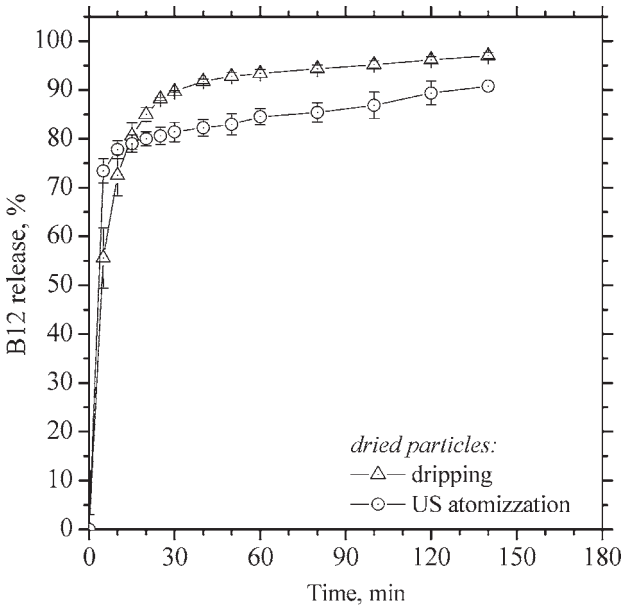


Fig. 7 – *B12 release profiles from dried chitosan particles (the error bars refer to the standard deviation SD)*

Conclusions

In this work the ultrasonic assisted atomization was applied as an encapsulation technique to obtain B12 loaded particles of chitosan. By this technique, funded on ionic gelation method, spherical microparticles of chitosan / B12 of about 600 micron (after drying treatment) were achieved.

Release tests demonstrated that the reticulation (ionic gelation) process was the key step in the structure particles formation, that, in turn, had a crucial role on the loaded particles release properties. In particular, the observed low encapsulation efficiency, low load and B12 fast release for both the two kinds of produced particles, i.e, millimetric and micro- spheres, suggested the presence of large pore sizes of the gel (PNMS) which take place according to the characteristics of used chitosan (degree of deacetylation) and the pH of the crosslinking environment. On the basis of correlation available in literature, an estimation of PNMS was done, obtaining radius of mesh sizes $\xi_{dripping}$ = 2.3 nm and $\xi_{atomization}$ = 1.2 nm more larger than the B12 Stokes radius (r_{Stokes} = 0.86 nm), confirming the above proposed hypotheses.

The main address derived from this work is that a controlled release from loaded chitosan particles can be achieved only tuning the polymeric network's structure. To this purpose new manufacturing protocols could be tested starting from chitosan features (DD, molecular weight) to reticulating conditions and stabilizing treatments that can affect structural properties such as crosslinking coadjuvant agents or thermal curing.

ACKNOWLEDGEMENTS

This work has been supported by the Italian Ministry of Education (PRIN 2008 – 2008HCAJ9T).

References

1. *Dalmoro, A., Barba, A. A., Lamberti, G., d'Amore, M.*, European Journal of Pharmaceutics and Biopharmaceutics **80** (2012) 471–477
2. *Dalmoro, A., Barba, A. A., Lamberti, G., Grassi, M., d'Amore, M.*, Advances in Polymer Technology (2012) in press
3. *Tran, V.-T., Benoît, J.-P., Venier-Julienne, M.-C.*, International Journal of Pharmaceutics **407** (2011) 1–11
4. *Gouin, S.*, Trends in Food Science & Technology **15** (2004) 330–347
5. *Benita, S.*, Microencapsulation: methods and industrial applications, Informa HealthCare, 2006
6. *Jyothi, N. V. N., Prasanna, P. M., Sakarkar, S. N., Prabha, K. S., Ramaiah, P. S., Srawan, G.*, Journal of microencapsulation **27** (2010) 187–197
7. *Freitas, S., Merkle, H., Gander, B.*, Journal of Controlled Release **102** (2005) 313–332
8. *Lyons, S., Wright, S.*, US6331317, (2003)
9. *Ré, M., Biscans, B.*, Powder Technology **101** (1999) 120–133
10. *Ensminger, D., Stulen, F.*, Ultrasonics: data, equations, and their practical uses, CRC Press, 2009
11. *Rajan, R., Pandit, A.*, Ultrasonics **39** (2001) 235–255
12. *Mason, T. J.*, Ultrasonics Sonochemistry **10** (2003) 175–179
13. *Liu, H.*, Science and engineering of droplets: fundamentals and applications, William Andrew, 2000

14. *Barba, A. A., d'Amore, M., Cascone, S., Lamberti, G., Titomanlio, G.*, Chemical Engineering and Processing: Process Intensification **48** (2009) 1477–1483
15. *Cascone, S., Lamberti, G., Titomanlio, G., Barba, A. A., d'Amore, M.*, Drug development and industrial pharmacy **38** (2012) 1486–1493
16. *Agnihotri, S. A., Mallikarjuna, N. N., Aminabhavi, T. M.*, Journal of Controlled Release **100** (2004) 5–28
17. *Sinha, V., Singla, A., Wadhawan, S., Kaushik, R., Kumria, R., Bansal, K., Dhawan, S.*, International Journal of Pharmaceutics **274** (2004) 1–33
18. *Park, K., Yeo, Y.*, US6767637, (2003)
19. *Rudzinski, W. E., Aminabhavi, T. M.*, International Journal of Pharmaceutics (2010)
20. *Dash, M., Chiellini, F., Ottenbrite, R. M., Chiellini, E.*, Progress in Polymer Science **36** (2011) 981–1014
21. *Albertini, B., Passerini, N., Rodriguez, L.*, Journal of Pharmacy and Pharmacology **57** (2005) 821–829
22. *Klaypradit, W., Huang, Y.*, LWT-Food Science and Technology **41** (2008) 1133–1139
23. *Pamujula, S., Graves, R. A., Moiseyev, R., Bostanian, L. A., Kishore, V., Mandal, T. K.*, Journal of Pharmacy and Pharmacology **60** (2008) 283–289
24. *Bodmeier, R., Oh, K.-H., Pramar, Y.*, Drug development and industrial pharmacy **15** (1989) 1475–1494
25. *Park, J., Saravanakumar, G., Kim, K., Kwon, I.*, Advanced drug delivery reviews **62** (2010) 28–41
26. *Bhumkar, D. R., Pokharkar, V. B.*, AAPS PharmSciTech **7** (2006) 138–143
27. *Lustig, S. R., Peppas, N. A.*, Journal of Applied Polymer Science **36** (1988) 735–747
28. *Amsden, B.*, Macromolecules **31** (1998) 8382–8395