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Original Article

CHITOSAN AS A POTENTIAL MICROENCAPSULATION CARRIER FOR ASCORBIC ACID STABILIZATION IN HETERODISPERSE SYSTEMS

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ABSTRACT

Objective: The purpose of this work is to assess the ionotropic gelation of chitosan as a new method to encapsulate and increase the stability of ascorbic acid (AA).

Methods: Chitosan was employed for the encapsulation of AA employing the technique of ionotropic gelation with sodium lauryl sulfate. The encapsulation process was made by two processes, homogenization and sonication-homogenization, respectively Ionotropic gelation was carried out by mixing chitosan and sodium lauryl sulphate solutions at 0.5, 1.0 and 1.5 % (w/v) concentrations with ~20 mg of AA.

Results: The two processes rendered spherical microcapsules with a narrow particle size distribution and particle size (0.7-2.1 µm), but only sonication-homogenization rendered less cohesive microcapsules. The encapsulation efficiency depended on the processing conditions and levels of parent materials and ranged from ~14 to 90% and ~14 to 72% for sonication-homogenization and homogenization, respectively. In both processes, runs with the lowest levels of chitosan (0.5%) were selected as optimal due to the spherical morphology, high encapsulation efficiency and less cohesive behavior. The addition of AA microcapsules into heterodisperse systems such as emulsions, semisolid systems and aqueous dispersions improved their thermal stability at 45°C rendering a shelf life (t_{90}) of 17.6, 21,1 and 3.3 days, respectively. Conversely, the products containing free AA had a shelf life of 1.8, 3.1 and 0.9 days, respectively.

Conclusions: The ionotropic gelation of chitosan with sodium lauryl sulfate improved the functionality, stability and shelf life of AA in heterodisperse systems.

Keywords: Microencapsulation, Ascorbic acid, Chitosan, Surfactant, Ionotropic gelation, Stabilization.

INTRODUCTION

Ascorbic acid (AA) is a vitamin required for the optimal functioning of metabolic processes in human beings and animals. It is also one of the most important antioxidants that can reduce the risk of cancer via several enzymatic mechanisms. However, humans need the exogenous administration of AA because they lack of the L-gluconolactone oxidase enzyme, which is responsible for its synthesis. If AA is applied topically, it contributes to the reduction of hyper pigmentation and wrinkle formation on the skin promoting the synthesis collagen. Moreover, it acts as an excellent antioxidant neutralizing the free radicals formed in the skin. These properties make AA an essential ingredient in most cosmetic and food products [1].

Unfortunately, in aqueous systems, AA readily undergoes an oxidative degradation, which is exacerbated by exposure to high temperatures, UV radiation and alkaline media containing O_2, Cu^{+2} or Fe^{+3} [2]. As a result, the development of hetero disperse topical formulations is very complex because some components in a formulation could also promote the degradation of AA causing detriment in the organoleptic properties of the product [3]. A feasible way to protect AA from the action of incompatible compounds and improve its stability is by microencapsulation with synthetic polymer barriers [4, 5, 6]. Several systems have been attempted to stabilize AA and guarantee its stability. For instance, liposomes produced with dipalmitoyl phosphatidyl choline and soy protein have improved its thermal stability [7, 8]. Further, niosomes formed with curcumin and quercetin has improved its antioxidant ability in food products [9]. On the other hand, solid-lipid microcapsules developed with palm fat reduced its degradation and masked its acidic taste [10]. Moreover, the coacervation of a w/o/w emulsion using corn oil, polyglycerol polyricinoleate, gelatin and gum arabic has reduced its degradation to a 35% at 37°C [11]. Conversely, spray-drying with Eudragit® has also been attempted, but degradation is still latent due to the presence of heat and the aqueous solution catalysis [12]. All these studies showed a minor increase in the stability of AA compared to its free form in solution. For this reason, new systems should be developed for its optimal stabilization.

Chitosan is a cationic and biodegradable polymer with good muco adhesive properties which make it ideal for the production of microcapsules by spray-drying, precipitation and ionotropic gelation [13,14]. The latter technique is considered the most practical since it does not involve the use of toxic solvents and present less scaled-up issues. One example is the ionotropic complexation of chitosan and tripolyphosphate encapsulating carvacol and acyclovir with efficiencies lower than 40% [15,16]. Moreover, spray-drying of the chitosan and tripolyphosphate complex has been attempted to encapsulate AA, but the properties of the resulting AA microparticles are highly dependent on the molecular weight, acetylation degree and spray-drying conditions [17, 18]. In order to avoid the problems associated with heat and the use of toxic solvents, this study explores the ionotropic complexation of chitosan with an anionic surfactant such as sodium lauryl sulfate. It is expected the high aqueous solubility of this surfactant as compared to tripolyphosphate will lead to a fast formation of resilient microcapsules of AA. To the best of our knowledge, there are no reported studies concerning the microencapsulation of AA using this type of complexation of chitosan with sodium lauryl sulfate. Therefore, the goal of this study is to develop AA microparticles by a complex ionotropic gelation and evaluate their stability in hetero disperse systems at 45°C compared to products containing the free form of AA.

MATERIALS AND METHODS

Materials

Ascorbic acid (lot D00030679) was obtained from Calbiochem (Darmstdat, Germany). Sodium lauryl sulfate (lot 128K0039) and chitosan (lot SLBH1773V, 25.4% acetylation degree, 552.1kDa molecular weight) were obtained from Sigma-Aldrich (S. t Louis, USA). Ethanol (99%, lot 0000416171) was purchased from Panreac Quimica (Barcelona, Spain).

Preparation of AA-loaded chitosan microcapsules by homogenization

Chitosan (CHI) dispersions of were prepared in 1% acetic acid. These dispersions and sodium lauryl sulfate solutions (SLS) were prepared at concentrations of 0.5%, 1.0% and 1.5% in nine different combinations. Subsequently, 10 mL of CHI containing ~20 mg of AA was added drop wise to a SLS solution under continuous stirring and homogenized at 7000 rpm (Ultraturrax, IKAT18, Campinas, Brazil) for 5 minutes.

Preparation of AA-loaded chitosan microcapsules by sonication-homogenization

The above procedure was employed to prepare the CHI and SLS solutions. Subsequently, 10 mL of SLS was placed in an ultrasonic bath (Ultrasonik TMN ey, USA) and 10 mL of chitosan dispersion was added drop wise for 1 h. Further, ~20 mg of AA was added and the resulting dispersion was sonicated for 1 h keeping the temperature below 40°C. The dispersion was then homogenized (Ultraturrax, IKAT18, Campinas, Brazil) at 7000 rpm for 5 minutes.

Encapsulation efficiency (EE)

The dispersions containing the microcapsules were centrifuged at 1600 rpmfor1.5h to precipitate the microcapsules. The supernatant was diluted in ethanol and UV absorbance was determined at 245 nm (HACH DR500, HACXH Company, Loveland, CO). A calibration curve was built at 2.5, 5.0, 10 and 20 µg/mL concentrations. The encapsulation efficiency (EE) was obtained from the equation:

EE = [(Total amount of AA – free AA)/ total amount of AA] x 100% Eq. 1

Morphology and particle size distribution

Microcapsules were dispersed in methanol, placed in glass slides and dried at room temperature. Micropictures were obtained on an optical microscope (BM180, BOECO, Germany) coupled to a digital camera (S8000fd, Fujifilm Corp., Japan) and taken at a 240X magnification. Particle size distribution was obtained from the microparticles counts using the Image]® (NIH, Bethesta, MD) and Minitab® (Minitab, Inc., State College, PA) softwares. The geometric mean diameters were determined from the cumulative frequency (probability scale) vs logarithm of mean diameter plots. The 50% cumulative frequency corresponds to the geometric diameter.

Preparation of hetero dispersed products containing AA microcapsules

The suspended microcapsules as obtained from runs F1, F2 and F6 of the method of sonication-homogenization were centrifuged (Precision Scientific Co., Chicago, USA) at 1600 rpm for 1.5 h, collected by vacuum filtration and dried at room temperature. Approximately, 2.82 mg, 282 mg, and 160 mg of encapsulated AA were added to the aqueous dispersion, emulsion and semisolid products, respectively. The composition of these formulations is listed in Table 1. The aqueous dispersion was prepared by adding the respective amount of microcapsules directly.

The semisolid dispersion was prepared by heating at 65°C and 60°C the aqueous and oil (olive oil) phases, respectively. Once the components in each phase were dissolved, the aqueous phase was added to the oil phase and homogenized for 10 minutes at 7000 rpm.

Subsequently, an aqueous dispersion containing the AA microcapsules was added when the temperature reached 30°C and a manual stirring with a flat spatula was continued until reaching room temperature. The emulsion system was prepared as described for the semisolid except that a homogenization time of 5 minutes was employed.

Table 1: Composition of the different products containing AA microcapsules

Type of product	Ingredient	Amount (w/w %)
Semisolid system	Water	83.6
	Cetiol OE®	2.0
	Cetyl alcohol	1.0
	Stearyl alcohol	1.0
	Emulgin® B2	1.0
	Isopropyl miristate	1.0
	Propilenglicol	4.0
	Sodium methyl paraben	0.4
	AA microcapsules	1.0
	Dimeticone®	1.0
	Xantham gum	1.0
	Olive oil	3.0
Emulsion system	AA microcapsules	5.0
	Olive oil	2.5
	Sodium lauryl sulfate	2.0
	Water	90.5
Aqueous dispersion	AA microcapsules	0.03
	Water	99.97

Stability studies of AA microcapsules

Aqueous suspensions, emulsions and hetero dispersed semisolid products containing AA microcapsules were stored on a convection oven at 45°C for two months. The concentration of AA in these products was assessed and compared with products containing equal amounts of free AA. All products were stored in tight plastic containers and protected from light. The aqueous dispersions were diluted with ethanol (1:2) and centrifuged at 1600 rpm for 15 minutes before measurement at 245 nm. On the other hand, emulsions were centrifuged at 1600 rpm for 20 minutes and microcapsules extracted from the aqueous phase, diluted with ethanol and analyzed for AA content. Conversely, ~ 1.5g of the semisolid product was taken and 20 mg of anhydrous sodium sulfate was added to ease the phase separation and centrifuged for 6 h at 1600 rpm. Subsequently, a dilution factor (0.25/12) was employed before measurement.

Statistical analysis

The analyses were performed by a completely randomized design. Data were tested by the analysis of variance (ANOVA) and Tukey's tests using the Design Expert® software 8.04 (Stat-Easy Inc., Minneapolis). The results were considered to be statistically significant when $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Production of AA microcapsules

Thehomogenization process rendered AA microcapsules with a different morphology and particle size depending on the CHI: SLS ratio employed (Fig. 1). For instance, F1and F2 runs, containing 0.5% w/v concentration of CHI rendered the largest number of microcapsules that were widely spread once dried, whereas high CHI levels larger than 1% w/v led to coalescence of particles

forming a continuous film with a rough surface due to their high surface energy.

These results are explained by the shear mechanism created during the homogenization process. In this case, the dispersions underwent shear when one portion of liquid traveled with a different velocity relative to the surrounding adjacent zone. This phenomenon occurs when the speed of the dispersion at the outside diameter of the rotor is higher than the one at the center once the dispersion exits the center from the closest clearance gap. In order to avoid further reduction of the particle size and achieve an optimum equilibrium between the formation and destruction of microcapsules the homogenization time was set to 5 minutes. Beyond this time, microparticles could be destroyed due to depolymerization of chitosan resulting in a reduction of particle size of microcapsules.

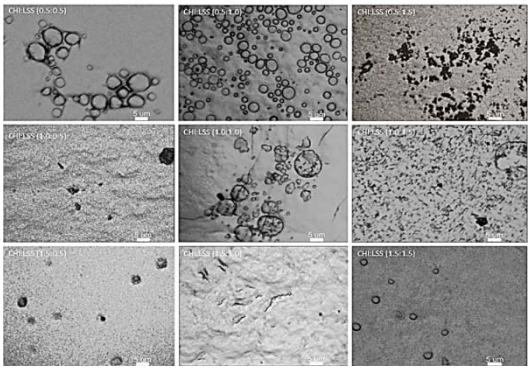


Fig. 1: Optical micropictures of AA microcapsules obtained by homogenization

On the other hand, the process of sonication-homogenization formed good microcapsules at low levels of CHI (i. e, 0.5%), especially for F1, F2 and F6 runs (Fig. 2). Sonication results from the utilization of sound energy to shake microparticles in an aqueous medium. Since the ultrasonic frequencies were >20 kHz, this process can also be considered ultrasonication. These frequencies induced cavitation alternating low and high-pressure waves in aqueous dispersions, leading to an intense break down of the large droplets produced initially when SLS and CHI were mixed. This mechanical breakdown is induced by strong hydrodynamic shear forces. As a result, layers of large droplets are removed to decrease the microparticles size rendering a large surface area. Therefore, the resulting cavitation is reduced to the level that no significant disaggregation of droplets occurred leading to the formation of microparticles of a constant size. Moreover, in the subsequent process of homogenization the high speed rotor drew the aqueous dispersions into the work head where they were thoroughly mixed and the centrifugal force drove the dispersions to the periphery of the work head and subjected them to a mechanical and hydraulic shear. Thus, microparticles were continuously forced through the stator screen at a high velocity and circulated back into the mix decreasing particle size quickly and forming a uniform microcapsule dispersion of sizes ≤2.1 µm.

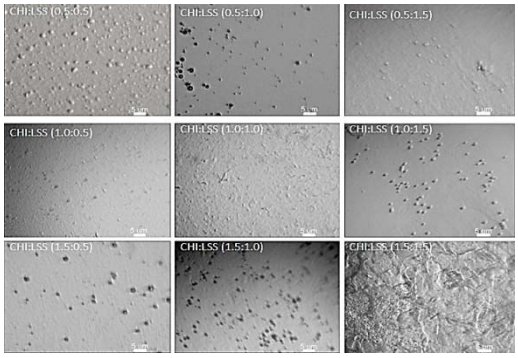


Fig. 2: Optical micropictures of AA microcapsules obtained byultrasonication-homogenization

The mean particle size and encapsulation efficiencies of AA is shown in table 2. The homogenization process from runs F1 to F4 rendered particles with a larger particle size than those produced by the sonication-homogenization process. The opposite case was observed from runs F5 to F9. This could be explained by the large concentration (>1%, w/v) of CHI which promoted the formation of large droplets, which in turn, were chopped down into small particles since the homogenization process took place resulting in particles of smaller sizes. On the contrary, the combined sonication-homogenization process caused a preliminary intense size reduction of these droplets even at high CHI levels and thus, the contribution of the homogenization process *per se on* the size reduction was very small. Further, a homogenization process lower than 5 minutes was employed to avoid intense particle size reduction and depolymerization of chitosan.

Table 2 also shows that the encapsulation efficiency (EE) ranged from ~14 to 90% and from 14 to ~70% when the sonication-homogenization and homogenization were employed, respectively. Further, in both processes the use of large levels of CHI (1.5%) was in detriment of the EE. Interestingly, runs that exhibited the largest presence of microcapsules such as F1, F2 and F6 also showed a high EE (> 56%). Therefore, it is desirable to use low levels of CHI (0.5%) to obtain the largest EE independent of the process employed. Conversely, the use of large levels of CHI promoted the coalescence of microparticles and the formation of a continuous layer with a rough surface. The yield loss in each process can be attributed to free AA dissolved in the aqueous media which was not incorporated into the microcapsules.

Thermal stability testing

A high temperature testing (accelerated testing) is commonly used as a predictor of long-term stability for drug and cosmetic products. Thus, most cosmetic companies follow the European Cosmetic Toiletry and Perfumery Association (COLIPA) guidelines conducting their accelerated testing at 45°C from 1 to 3 months. Thus, it is known if a cosmetic product is stored at 45°C for three months and exhibits acceptable stability it should be stable at room temperature for two years.

Table 2: Encapsulation efficiency and particle size for each experimental run

Run	Chitosan (w/v %)	Sodium lauryl sulfate (%)	Encapsulation efficiency (%)		Particle size (µm)	
			H	S-H	H	S-H
F1 (0.5:0.5)	0.5	0.5	71.3	76.9	1.8±1.0	0.9±0.1
F2 (0.5:1.0)	0.5	1.0	65.4	63.5	2.1±0.0	1.2±0.0
F3 (0.5:1.5)	0.5	1.5	14.2	46.9	1.3±0.0	0.9±0.1
F4 (1.0:0.5)	1.0	0.5	39.9	63.2	1.1±0.1	0.7±0.0
F5 (1.0:1.0)	1.0	1.0	15.6	74.8	1.0±0.0	1.2±0.1
F6 (1.0:1.5)	1.0	1.5	55.9	88.7	0.8±0.0	1.5±0.0
F7 (1.5:0.5)	1.5	0.5	32.5	52.2	0.8±0.0	0.8±0.0
F8 (1.5:1.0)	1.5	1.0	28.7	59.2	1.1±0.0	2.0±0.1
F9 (1.5:1.5)	1.5	1.5	15.2	14.5	1.2±0.1	1.4±0.1
		p-value	0.044		0.744	

H: Homogenization; S-H: Sonication-homogenization.

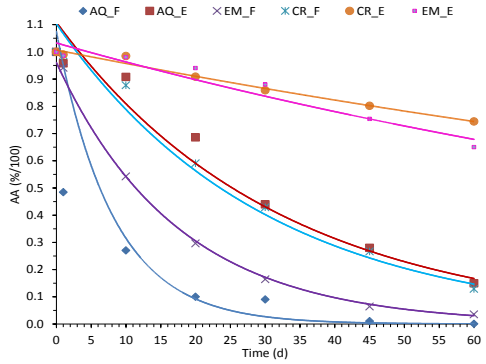
Table 3: First order parameters and shelf-life of AA in different heterodisperse systems

Product	First order parameters				$k_{F/E}$	Shelf life, t_{90} (days)	
	ENC		Free			ENC	Free
	k (d^{-1})	r^2	k (d^{-1})	r^2			
Aqueous dispersion	0.032	0.9765	0.123	0.9181	3.8	3.3	0.9
Emulsion	0.006	0.9117	0.057	0.9962	9.5	17.6	1.8
Semisolid	0.005	0.9867	0.034	0.9833	6.8	21.1	3.1

ENC: AA Encapsulated; k: degradation constant.

The thermal stability profiles were determined for products containing AA microcapsules and compared to those having the free acid (Fig. 3, table 3). The stability profiles showed a first order degradation exhibiting an exponential decay (r^2>0.91). Products containing microencapsulated AA exhibited smaller degradation constant values (k) than those containing the free acid having from a 3.8 to 9.5 fold increase in stability.

The best results were obtained for the semisolid system, which retained 90% of the initial concentration within 21 days at 45°C. The degradation process was accelerated when AA microcapsules were suspended in aqueous media due to the rapid shell swelling allowing for a fast release of AA exposing this acid to a drastic hydrolytic degradation.



AQ_F: Aqueous dispersion with free AA; AQ_E: Aqueous dispersion with encapsulated AA; EM_F: Emulsion with free AA; EM_E: Emulsion with encapsulated AA; CR_F: semisolid product with free AA; CR_E: Semisolid product with encapsulated AA

Fig. 3: Accelerated stability testing performed at 45°C of different products containing AA

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CONCLUSION

The morphology, particle size and encapsulation efficiency of AA microcapsules depended on the processing conditions and level of chitosan employed. In both processes, the best AA microcapsules were obtained at low levels of chitosan. The encapsulation process greatly enhanced the stability of AA microcapsules, especially in emulsion and semisolid systems. These results can be useful during the drug development stage to predict the desired ambient storage conditions for products containing AA microcapsules.

CONFLICT OF INTERESTS

Declared None

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