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Chitosan/cashew gum nanogels for essential oil encapsulation

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ABSTRACT

Nanogels based on chitosan and cashew gum were prepared and loaded with *Lippia sidoides* oil. Several parameters such as cashew gum concentration and relative oil content in the matrix had their influence on nanogel properties investigated. Nanogels were characterized regarding their morphologies, particle size distributions, zeta potential, Fourier transform infrared spectroscopy and essential oil contents. The release profile was investigated by UV/vis spectroscopy and its efficacy was determined through bioassays. Results showed that samples designed using relative ratios matrix:oil 10:2, gum:chitosan 1:1 and 5% gum concentration showed high loading (11.8%) and encapsulation efficiency (70%). Nanogels were found to exhibit average sizes in the range 335–558 nm. *In vitro* release profiles showed that nanoparticles presented slower and sustained release. Bioassays showed that larval mortality was related mainly to oil loading, with samples presenting more effective larvicide efficacies than the pure *L. sidoides* oil.

1. Introduction

Hydrogel particles in the nanometer range are called nanogels, being usually formed, for example, by physical interaction of oppositely charged ions, such as chitosan, a polycation and alginate, a polyanion. These systems have many applications, particularly in the pharmaceutical and medical fields, mainly as a consequence of the fact that they can entrap in their nanogel network drugs and bioactive substances and thereby improving their efficacy, physical stability and release properties (Carvalho, Goncalves, Gil, & Gama, 2007; Heyden, Babooram, Ahmed, & Narain, 2009; Kettel, Dierkes, Schaefer, Moeller, & Pich, 2011; Nukolova, Yang, Kim, Kabanov, & Bronich, 2011; Ramos et al., 2011; Sasaki, Tsuchido, Sawada, & Akiyoshi, 2011; Yu, Li, Qiu, & Jin, 2008; Zhang, Oh, Allen, & Kumacheva, 2004).

In the last decade, different nanoparticles (NPs) based on polysaccharides were produced for drug delivery applications (Berger, Reist, Mayer, Felt, & Gurny, 2004; De & Robinson, 2003; Tan, Chan, & Heng, 2005; Zheng, Gao, Zhang, & Liang, 2004). These systems can also be applied in the agricultural field, where pesticides can be entrapped in the polymeric matrix, maximizing their effect, at low concentration (Soppirmath & Aminabhavi, 2002). Alginate and chitosan are the most used polysaccharides for NP preparations. These NPs are of particular interest due to the fact that they can be obtained from biopolymers which are biodegradable and biocompatible. Moreover, their preparation methods involve easily handling in aqueous medium, thereby avoiding the use of environmentally impacting organic solvents. Chitosan (CH), a D-glucosamine and N-acetyl-D-glucosamine linked by beta (1–4) glycosidic bond polymer, is a deacetylated form of chitin, an abundant polysaccharide present in crustacean shells (Berger et al., 2004; Muzzarelli et al., 2012; Tan et al., 2005). Nanoparticles having hydrodynamic diameters of 400 nm, made of chitosan and alginate gels were used for encapsulating an anticancer agent, aiming at colon targeting (Laroui et al., 2010). The nanogel was reported to collapse on the colon area, releasing the active component and hence resulting in the expected healing effect.

In another approach, NPs obtained by polyelectrolyte complex formation of chitosan and poly-gama-glutamic acid were shown to have average hydrodynamic diameters in the range from 150 nm to 330 nm, which were dependent on concentration and polymers ratio as well as on pH medium (Hajdu et al., 2008).

Cashew gum is an exudate from *Anacardium occidentale* tree and has similar properties to those of Arabic gum, whereby their structures have a main chain of galactose units, having branches of arabinose, glucose and rhamnose. Uronic acid units were also found to be present in side chains (de Paula, Heatley, & Budd, 1998). Cashew gum nanoparticles were obtained by free radical polymerization of acrylic onto cashew gum backbone, resulting in particle sizes in the range from 71 nm to 420 nm (da Silva, Feitosa, Paula, & de Paula, 2009). On another approach, nanoparticles of chitosan and cashew gum were produced in aqueous medium by ionic complexation (Oliveira, Ciarlini, Feitosa, de Paula, & Paula, 2009). *Lippia sidoides* is a plant native of Brazilian Northeast region and its leaves contains an essential oil rich in thymol which has fungicide

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and bactericide activities (Camurça-Vasconcelos et al., 2007). The oil has been demonstrated to have also a larvicide effect against larvae of *Aedes aegypti* (Carvalho et al., 2003), the dengue vector which is responsible for many deceases and even deaths in tropical countries such as Brazil. Arabic gum has been used as an essential oil encapsulating agent (Fernandes et al., 2008), using high oil and polymer contents. This oil has also been recently encapsulated in chitosan/cashew gum based beads obtained by a coacervation method (Paula, Sombra, Cavalcante, Abreu, & de Paula, 2011), with low loadings, ranging from 2.4% to 4.4%. Aiming to improve essential oil loading and release profiles, our research group decided to fully investigate a new matrix composed of chitosan and cashew gum as an encapsulating agent for *L. sidoides*. The development of polymeric matrices for encapsulation of a natural essential oil with larvicide activity, would favor efficacy, associated with safety, in the handling to the environment. Hence, this work reports on the preparation by spray drying of chitosan–gum nanoparticles loaded with *L. sidoides* leaves oil, as well as on the investigation of the effects of polymer concentration, chitosan–gum relative ratio on the nanoparticles particle size and encapsulation efficiency. *In vitro* release studies and *in vivo* experiments were also carried out.

2. Experimental

2.1. Materials

Chitosan (75% deacetylation degree, $M_w = 1.8 \times 10^5\ g\,mol^{-1}$) were supplied by a local industry in CE, Brazil. Cashew gum extracted from native trees from Ceará ($M_w = 1.1 \times 10^5\ g\,mol^{-1}$) was purified as described in a previous work (Paula, Gomes, & de Paula, 2002). *L. sidoides* oil (Produtos Naturais LTDA – Pronat Horizonte, CE, Brazil) and emulsifier Tween 80 (VETEC, SP, Brazil) were used as received.

2.2. Preparation of chitosan–gum nanogels

Solutions of 1%, 5% and 10% (w/v) of cashew gum and chitosan were prepared using relative ratios of gum:chitosan = 1:10, 1:2, 1:1, 2:1 and 10:1. Chitosan solutions were prepared by dissolving the desired amount of chitosan in 1% acetic acid solution. Cashew gum solutions were prepared by dissolving the gum in deionized water to the desired concentration.

Polymer complexes of gum:chitosan were prepared by adding the cashew gum solution to chitosan solution, by a peristaltic pump, using a flow rate of $1.0\ ml\,min^{-1}$. An emulsion was separately prepared using oil and Tween and slowly added to the polymer complexes using different polymer matrix:oil ratios (10:1, and 10:2, w/w). The turbid solution was then spray dried in a Buchi equipment, model B290, operating at inlet temperature 170 °C, outlet temperature 65 °C, pump feed flow $5\ ml\,min^{-1}$, air volume flow $35\ m^3\,h^{-1}$ and aspirator flow meter $841\,h^{-1}$. Table 1 shows the experimental variables of the gum:chitosan nanogel preparation. A total of 11 runs in duplicate were performed.

2.3. Nanogel characterization

Gum–chitosan nanogels were characterized by FTIR infrared spectroscopy in a Shimadzu spectrometer, model 8300. Particle size and distribution and zeta potential were determined by photon correlation spectroscopy (PCS) in a Zetasizer Malvern, model Zen 3500.

Essential oil loading was determined by UV/vis spectroscopy (Paula et al., 2011), at 260 nm (Micronal, model B582) as following: a 10 mg sample was crushed in ethanol and its concentration was calculated using a calibration curve (Eq. (1)). Data obtained were confirmed by gas chromatography-GC in a Shimadzu equipment, model GC 17A.

Table 1
Matrix containing the variables and the ratios studied for production of gum:chitosan (CG:CH) nanoparticles.

Run	Gum concentration (%)	Matrix:oil	CG:CH ratio
1	1	10:1	10:1
2	5	10:1	10:1
3	10	10:1	10:1
4	1	10:2	10:1
5	5	10:2	10:1
6	10	10:2	10:1
7	1	10:1	2:1
8	1	10:1	1:1
9	1	10:1	1:2
10	1	10:1	1:10
11	5	10:2	1:1

$$Abs = 0.0030 - 0.0150\ conc \quad (1)$$

where *Abs* is the absorbance and *conc* is the oil concentration in ppm. This equation was found to have a correlation coefficient, $R^2 = 0.998$.

Oil encapsulation efficiency (EE) was determined using Eq. (2):

$$EE\,(\%) = \frac{M}{M_o} \times 100 \quad (2)$$

where M is the amount (mg) of oil in loaded sample as determined from Eq. (1) and M_o is the initial oil amount (mg) added to the emulsion.

In vitro release was conducted by dissolving 100 mg sample in 10 ml distilled water and the resulting solution placed in a dialysis bag which was kept in a beaker containing 200 ml distilled water, under constant temperature and stirring. At regular time intervals, an aliquot of 1.0 ml was withdrawn, diluted and analyzed by UV/vis. Medium volume was replaced to its original value. All measurements were replicated twice and data were averaged, using a 95% confidence level.

In vivo experiments were carried out in order to verify the efficacy of *L. sidoides* oil larvicide, where 12 and 24 mg of nanoparticles were placed in a 50 ml Becker containing 20 third instar *St. Aegypti* larvae provided by the Ceará State Health Secretary. Larvae mortality was determined after 24 h, 48 h and 72 h, by counting dead specimens which were subsequently removed (Paula et al., 2011). A control (blank) sample was used. All experiments were carried out three times and data averaged.

3. Results and discussion

Nanoparticles were prepared using cashew gum and chitosan solutions at different polymer concentrations (1–10%, w/v) and different polymer matrix:oil ratio (w/w) (10:1 and 10:2). It was maintained initially a relative ratio gum:chitosan 10:1, in order to observe the effects of the polymer concentration and the oil content on the encapsulation efficiency. The operational spray drying yield varied from 45% to 60%, at the best thermal efficiency and powder recovery for the designed formulations.

3.1. Nanogel characterization

The structural characterization obtained by FTIR spectroscopy for loaded nanogel nanoparticles is shown in Fig. 1(I).

All samples presented cashew gum absorption bands corresponding to COO^- groups and water at $1645\ cm^{-1}$. Chitosan main vibrational groups were present as the C—N bond stretching at $1372\ cm^{-1}$, and the chitosan symmetric and asymmetric amino stretching bands are also shown at $1410\ cm^{-1}$, $1565\ cm^{-1}$ and

Table 2
Nanoparticles composition, loading, encapsulation efficiency (EE%), particle size and polydispersity index (PDI) for nanoparticles produced with gum:chitosan ratio 10:1.

Run	Cashew gum (%)	Matrix:oil	Loading (%)	EE (%)	Particle size (nm)	PDI
1	1	10:1	6.7 ± 0.5	61	335 ± 116	0.502
2	5	10:1	5.5 ± 0.7	55	385 ± 45	0.365
3	10	10:1	6.0 ± 1.3	65	555 ± 153	0.613
4	1	10:2	6.5 ± 0.5	60	558 ± 116	0.524
5	5	10:2	11.0 ± 1.0	70	405 ± 52	0.467
6	10	10:2	8.0 ± 1.0	40	551 ± 106	0.554

1640 cm^{-1}, respectively (Lawrie et al., 2007; Paula, de Paula, & Bezerra, 2006). By increasing chitosan in the formulation, there was an increase of the amino stretching at 1410 cm^{-1}. *L. sidoides* essential oil main absorption bands, at 1622 cm^{-1}, 1421 cm^{-1}, and 1100 cm^{-1} assigned to thymol aromatic groups were found to be overlapped with matrix polymer groups. Evidence of cashew gum and chitosan interaction was detected at 1563 cm^{-1}, in good agreement with literature (Paula et al., 2002).

Particle size distribution was analyzed for loaded gum–chitosan nanogel nanoparticles. Fig. 1(IIa) shows the particle size distribution for samples gum:chitosan 10:1 produced with 1%, 5% and 10% cashew gum polymer concentration. It can be seen that all samples presented unimodal distributions and polydispersity indexes varying from 0.365 to 0.613. At 1% gum concentration, oil content does not influence size distribution, unlike 5% gum concentration, whose PDI increases with M:oil ratio.

As a general trend, it seems that size distribution becomes broader as cashew gum concentration is increased.

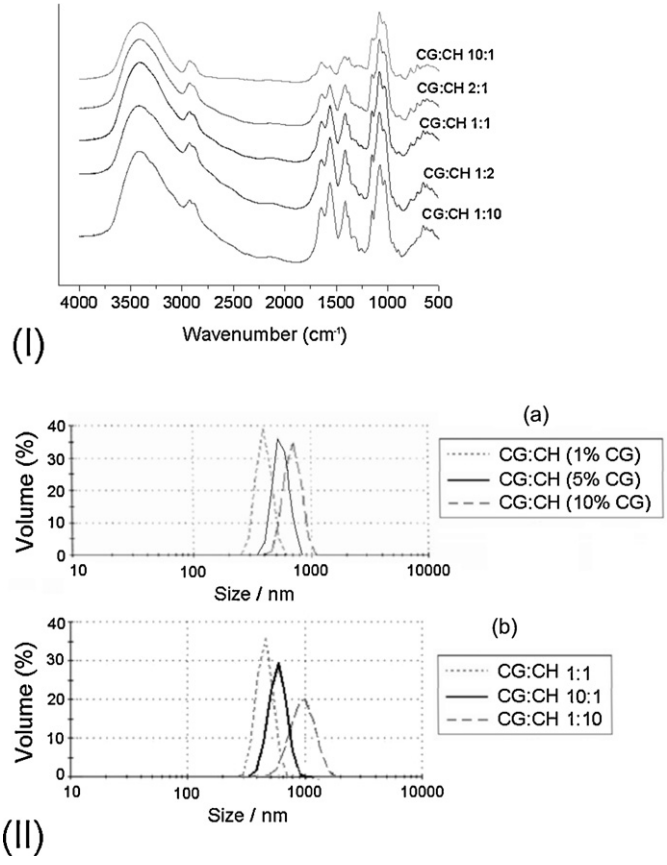

Fig. 1. (I) Infrared spectra of cashew gum–chitosan nanoparticles with different gum:chitosan (CG:CH) relative ratios. (II) Particle size distribution of the gum:chitosan (CG:CH) 10:1 nanoparticles at (a) different cashew gum concentrations (% CG) and (b) several gum:chitosan (CG:CH) relative ratios.

Fig. 1(II b) shows the particle size distribution for nanogel nanoparticles produced with polymer concentration (cashew gum) of 5% and different gum:chitosan ratios. A trend can be observed, whereby on increasing the chitosan content in the matrix, larger particle were obtained, exhibiting values as high as 899 nm. Moreover, size distributions becomes narrower as the chitosan proportion is increased. This has also been observed for chitosan–polyglutamic acidic matrixes (Hajdu et al., 2008).

A likely explanation is that the increase of chitosan proportion in matrix leads to a reduction in the gum–chitosan interactions and consequently favors the formation of particles bonded by hydrogen chitosan interactions between their chains (interchain interactions). The positive amino group of chitosan causes repulsion between the chains, leading to high nanoparticle sizes.

3.2. Nanogel morphology

Nanogel morphologies were analyzed by Scanning Electron Microscopy – SEM, by placing a drop of nanoparticle sample on carbon stickers on aluminum stubs, drying and coated with gold, prior to visualization in a Olympus equipment. Data obtained (not shown) revealed that most particles exhibit spherical shapes, either free or in clusters of aggregates. Upon spray drying, some particle aggregation has been observed, leading to the formation of large clusters, a phenomena that is usual for systems such as Gum arabic–maltodextrin nanoparticles (Peres et al., 2011).

3.3. Effect of polymer concentration

The effect of polymer concentration on the loading and particle size of the nanogel nanoparticles was investigated. Table 2 shows the data obtained. Gum:chitosan 10:1 nanogels presented loading varying from 5.5% to 11.0%. The increase of the polymer concentration (cashew gum) in the matrix did not affect significantly the loading of the gum:chitosan nanoparticles produced with low oil content (matrix:oil 10:1). On the other hand, for samples produced with higher oil content (matrix:oil 10:2) it was observed an increase (up to 11%) in the loading (for cashew gum 5% concentration). The encapsulation efficiency was found to slightly increase with polymer concentration, for both matrixes (matrix:oil 10:1 and matrix:oil 10:2), being in the range from 60% to 70%, for matrix:oil 10:2, at gum concentration from 1% to 5%. Particle size was shown to augmenting with polymer concentration for matrix:oil 10:1 and remains fairly constant for matrix:oil 10:2. Higher loading was obtained by encapsulating *L. sidoides* essential oil in beta-cyclodextrin, resulting in oil content of 11.7%. No particle size was reported. (Fernandes, Ehen, Moura, Novak, & Sztatisz, 2004).

No noticeable trend was observed for NPs polydispersity indexes (PDI), however the least polydispersion is exhibited by samples prepared using 5% gum concentration, for both low an high oil levels (runs 2 and 5). Particles produced by spray-drying technique tend to reach high particle size when formulated from solutions with high solid content, due to the likely possibility of agglomeration of powder particles. From Table 2, it can be inferred

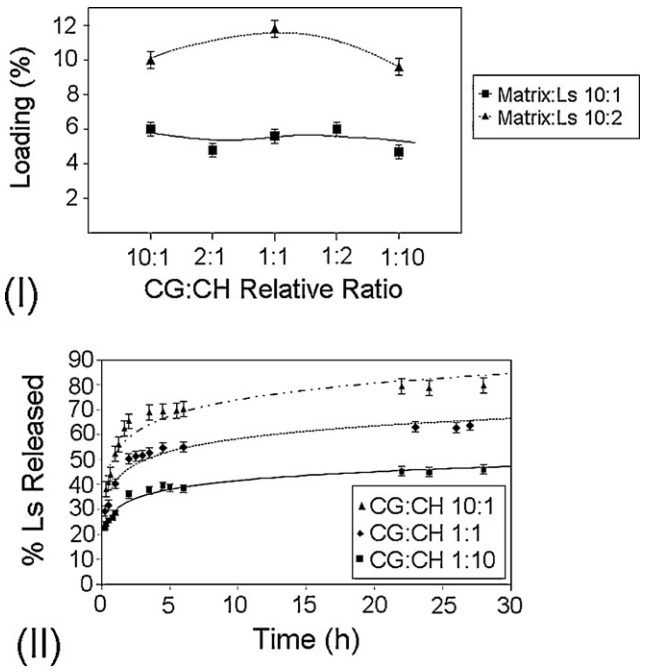

Fig. 2. (I) Nanoparticles loading as function of gum:chitosan (CG:CH) relative ratio with different oil (Ls) content in the matrix (matrix:Ls) for samples with 5% gum concentration. (II) *Lippia sidoides* essential oil (Ls) *in vitro* release from nanoparticles with gum:chitosan (CG:CH) relative ratios 1:10, 1:1 and 10:1, matrix:oil 10:2 and 5% gum concentration.

that the parameters used in run 5 were optimized regarding loading and EE (12% and 70%, respectively).

3.4. Effect of gum:chitosan relative ratio

The polymer concentration (5% cashew gum) was kept constant, aiming at evaluation of the effect of gum:chitosan relative ratio and the oil content on nanoparticles properties. Nanogel loading as a function of the gum:chitosan relative ratio and the oil content in the matrix is shown in Fig. 2(I).

Oil loading was found not to vary significantly with the increase of chitosan in the matrix, for the samples produced with low oil content (matrix:oil 10:1). This samples showed a maximum loading of 6.5%; however, with higher initial oil content (matrix:oil 10:2) it was observed that nanogels exhibit higher loading capacities, with values of 11.8% and 10.0%, for samples gum:chitosan 1:1 and gum:chitosan 1:10, respectively.

3.5. Zeta potential analysis

Zeta potential of gum:chitosan nanogel nanoparticles were determined for samples produced with high polymer concentration (5–10%), different gum:chitosan relative ratio and oil contents in the matrix. Results showed that the oil content in the matrix influenced neither the size nor the zeta potential, where formulations differing only in the oil content presented almost identical results (data not shown). Therefore, it was decided to investigate the particle size and the zeta potential for samples produced with low polymer concentration (1% cashew gum), matrix:oil 10:1 and different relative gum:chitosan ratios. Table 3 shows the data obtained.

It can be seen that particle sizes are in the range 335–899 nm, mostly having unimodal distribution; however one sample (gum:chitosan 2:1) presented bimodal distribution with particle of diameter of 21 nm (80% by volume), and the other with diameter of 456 nm (20% by volume).

Table 3
Particle size, polydispersion index (PDI) and zeta potential for nanoparticles produced with cashew gum 1% and matrix:oil ratio 10:1.

Run	Gum:chitosan ratio	Particle size (nm)	PDI	Zeta potential (mV)
1	10:1	335 ± 116	0.502	4.0 ± 0.8
7	2:1	456 ± 10 (20%)[a]	0.408	17.0 ± 2.6 (20%)[a]
		21 ± 10 (80%)[a]		38.0 ± 1.8 (80%)[a]
8	1:1	391 ± 55	0.439	39.2 ± 0.9
9	1:2	390 ± 89	0.373	36.3 ± 3.0
10	1:10	899 ± 21	0.633	49.6 ± 1.3

[a] Percentage of particle volume.

Relative polymer charges were determined by zeta potential measurements. The gum–chitosan nanogel nanoparticles presented positive potential for all formulations, with values ranging from +4 mV to +49 mV. Chitosan present high quantity of protonated amino groups (75% deacetylation degree); whereas cashew gum present around 5% of carboxylic acid groups due to the presence of uronic acid residues. The complexation of chitosan and cashew gum generated nanoparticles with positive potential, where the lowest value was reached for gum:chitosan 10:1 (4 mV) and the highest for gum:chitosan 1:10 (49 mV). Data suggests that chitosan molecules are located at the NP surface, even for high gum:chitosan ratio and contributes more effectively to nanogel stabilization.

Carvacrol, one of the *L. sidoides* terpenes, was encapsulated by chitosan, presenting encapsulation efficiency of 14–31% and loading in the range 3–21%, with particle sizes in the range 40–80 nm, and zeta potential values of 25–29 mV (Keawchaoon & Yoksan, 2011).

The best results regarding to maximum loading, encapsulation efficiency, lower particle size were obtained with formulations produced with higher oil content in the matrix (matrix:oil 10:2) associated with a 5% cashew gum polymer concentration. In this sense, the *in vitro* release profile of the nanogels produced at those conditions were performed, investigating the effect of the gum:chitosan relative content in the release profile of *L. sidoides* essential oil.

3.6. In vitro release

In vitro release studies on nanogels gum:chitosan 1:10, 1:1 and 10:1 produced at 5% cashew gum concentration, matrix:oil 10:2 were performed, and the results are shown in Fig. 2(II).

Release pattern showed a difusional profile, dependent of the gum:chitosan ratio. After 3 h, the nanogels presented 30%, 50% and 65% of oil released for samples gum:chitosan 1:10, gum:chitosan 1:1 and gum:chitosan 10:1, respectively. The increase of cashew gum in the polymer matrix, which resulted in an increase of nanogel hydrophilic character, seemed to favor the release of *L. sidoides* essential oil. After 24 h, it is observed that sample gum:chitosan 10:1 showed 74% of oil released. Reducing the quantity of cashew gum in the matrix, slower release profiles were achieved, where gum:chitosan 1:10 sample presented the lowest percentage of oil released (35%).

The nanogel release profile was determined using Eq. (3):

$$\frac{M_t}{M_\infty} = K\,t^n \tag{3}$$

where M_t/M_∞ denotes the fraction of oil released, t is the release time, and K represents a constant characteristic of the system (Paula et al., 2006; Soppirmath & Aminabhavi, 2002). The diffusion exponent (n) is an indication of the mechanism of oil release and takes values depending on the geometry of the release device. A linear form of Eq. (3) can be obtained by plotting $\ln M_t/M_\infty$ against $\ln t$,

Table 4
Release kinetic parameters (n and K) for the gum:chitosan nanoparticles.

Gum:chitosan	n	K	R^2
1:10	0.1904	0.6350	0.991
10:1	0.3203	0.6544	0.994
1:1	0.3091	0.6114	0.993

whose angular coefficient is n and the linear coefficient is K. Table 4 shows data obtained.

The n exponent for all samples was below 0.5, duly pointing out to a Fickian behavior (case I transport), where the release is dictated by a diffusional behavior.

3.7. Bioassays

Gum:chitosan nanogels loaded with *L. sidoides* essential oil were evaluated regarding their mortality kinetics against third instar *St. aegypti* larvae. Nanogels prepared under different reactions conditions were tested, using samples weighting 12 mg and 24 mg, produced with gum:chitosan 10:1, at different cashew gum concentrations and matrix:oil contents. Tests conducted using 12 mg of the sample (data not shown) presented no significant mortalities, showing maximum mortality values of 30%, after 72 h. On the other hand, data on larvae mortality using 24 mg of the sample, presented markedly visible differences, as shown in Fig. 3(I).

It can be seen that the increase in the larval mortality was proportional to the cashew gum and oil content in the matrix. Best results were obtained for sample prepared using 5% cashew gum concentration, with mortality rates of 55% and 87%, after 24 h and 48 h, respectively, and for sample prepared using 10% cashew gum concentration, resulting in 100% of mortality, after 24 h. Table 5 shows the loading values and corresponding oil concentration in the aqueous medium, for 24 mg nanoparticle samples.

The LC_{50} (lethal concentration for killing 50% larvae) for *L. sidoides* free oil is 36 ppm. Comparing to the oil concentration encapsulated in the nanoparticles, it can be seen that only the 5% cashew gum and 10% cashew gum samples, present higher oil concentration (38 ppm and 53 ppm, respectively) than the required to cause 50% of larval mortality. In fact, these two formulations are the ones that exhibit highest mortalities.

Bioassays were also conducted for nanogels prepared with different gum:chitosan relative ratios, at 5% cashew gum concentration. Nanogels produced using matrix:oil 10:1 presented loading values around 6%, with a corresponding concentration of 14 ppm and 28 ppm of *L. sidoides* oil (sample masses of 12 mg and 24 mg, respectively). Data revealed that even for high sample amount, mortality values did not reach values beyond 35%, no matter what the gum:chitosan relative ratio (data not shown). On the other hand, nanogels produced using higher oil content (matrix:oil 10:2), presented significant differences on mortality values according to sample amount and gum:chitosan relative ratio, as shown in Fig. 3(II). It can be seen that for 12 mg samples (Fig. 3IIa), highest mortality was achieved for sample gum:chitosan 1:10 (oil content of 24 ppm), while sample gum:chitosan 10:1 (oil content of 12 ppm) presented the lowest figure, even after 72 h. It can be depicted that

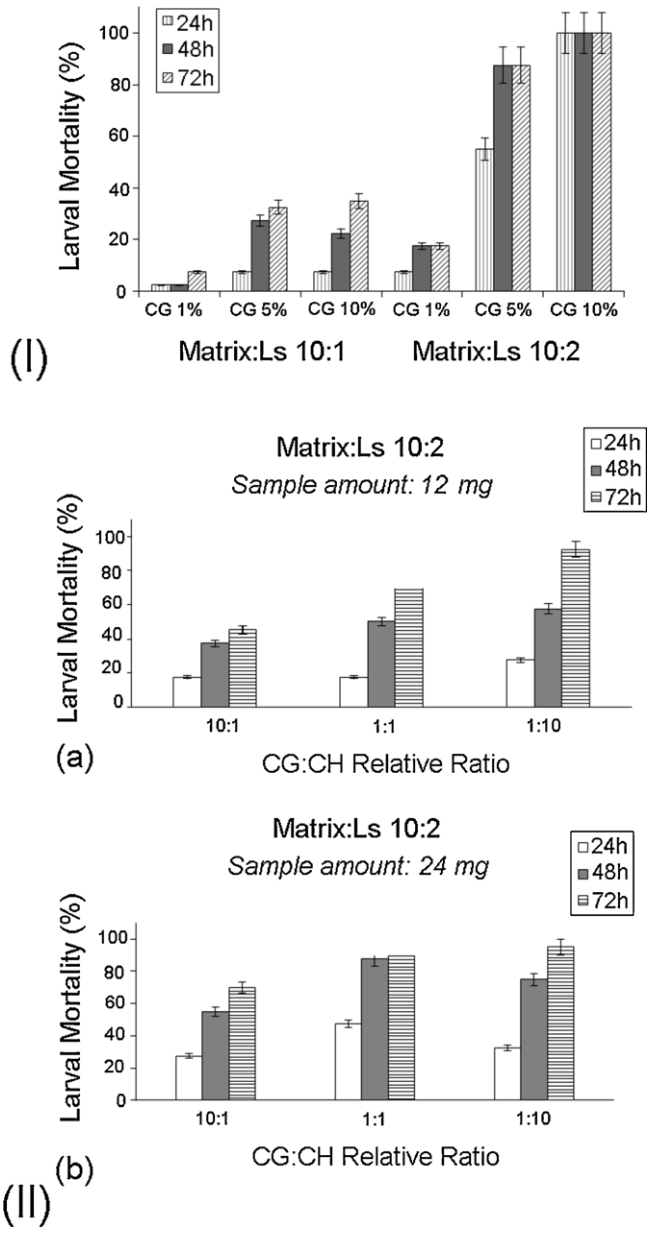

Fig. 3. (I) Larval mortality using 24 mg of samples produced with gum:chitosan (CG:CH) 10:1, at different gum (CG) concentrations and matrix:oil (matrix:Ls) contents. (II) Larval mortality of samples produced with matrix:oil (matrix:Ls) 10:2, cashew gum (CG) 5%, and different gum:chitosan (CG:CH) relative ratio: (a) 12 mg and (b) 24 mg sample amount.

nanogel with high chitosan content (gum:chitosan 1:10) seems to exhibit a oil sustained release, which is corroborated by its low value of diffusion exponent (n = 0.1904) given in Table 4. Nanogel particle size seem not to have a noteworthy effect on larvae mortality, in the size range investigated (335–899 nm). On the other hand,

Table 5
Loading values and the corresponding oil concentration for gum:chitosan ratio 10:1 nanoparticles in the aqueous medium.

Cashew gum concentration (%)	Matrix:oil 10:1		Matrix:oil 10:2	
	Loading (%)	Oil concentration (ppm)	Loading (%)	Oil concentration (ppm)
1	6.7 ± 0.5	32	6.5 ± 0.5	30
5	5.5 ± 0.7	26	11.0 ± 1.0	53
10	6.0 ± 1.3	29	8.0 ± 1.0	39

tests using 24 mg of nanogels (Fig. 3IIb) revealed that gum:chitosan 1:1 and gum:chitosan 1:10 samples (oil contents of 58 and 48 ppm, respectively) display nearly the same larvae mortalities (over 90%), in good agreement with the fact that these samples have oil loading in the range 10.0–11.8%.

It can be inferred that the aforementioned samples presented high ability to encapsulate and retain the *L. sidoides* essential oil inside the polymeric chains and therefore maintaining the larvicide effect, reaching mortality rates superior to that obtained by the *L. sidoides* pure oil.

4. Conclusions

The nanoencapsulation of *L. sidoides* essential oil by spray drying under different conditions was investigated regarding to the relative gum:chitosan composition, the cashew gum concentration and matrix:oil relative content. The loading and encapsulation efficiency of *L. sidoides* oil were optimized, for samples produced with matrix:oil 10:2 using a 5% cashew gum concentration. Particularly, sample gum:chitosan 1:1 presented highest loading (11.8%) and encapsulation efficiency (70%). FTIR showed the presence of chitosan, cashew gum and thymol in the nanoparticles. Most nanogel nanoparticle sizes ranged from 335 nm to 558 nm, mainly with unimodal distribution and positive potential zeta values. The increase of chitosan in the matrix led to particles with high size, probably due to the abundant positive amino groups in the nanoparticle, which caused electrostatic repulsion between the chains. *In vitro* release profiles revealed markedly Fickian behavior; a prolonged release being obtained for the sample with larger chitosan proportion, i.e., gum:chitosan 1:10. All the nanogels produced presented efficacy against *St. aegypti* larvae, where the mortality rate was related to the loading values and gum:chitosan ratios. In particular, samples gum:chitosan 1:1 and gum:chitosan 1:10 showed respectively 87% and 75% of mortality after 48 h, reaching over 90% of mortality at 72 h. These results showed that the gum–chitosan nanoparticles were designed and present sustained release features.

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