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Microencapsulation of a Colombian *Spodoptera frugiperda* Nucleopolyhedrovirus with Eudragit® S100 by Spray Drying

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ABSTRACT

A Colombian Spodoptera frugiperda nucleopolyhedrovirus NPV003 with high potential for the development of an efficient biopesticide was microencapsulated by spray drying with a pH dependent polymer (Eudragit® S100). Conditions for microparticles production were standardized and microencapsulation process was validated. Physical properties, insecticide activity and photo-stability of microencapsulated virus were determined. The microparticles were spherical and irregular shaped, with sizes between 17.64 and 19.47 µm. Moisture content was 10.38 ± 0.87%; encapsulation efficiency 84.61± 13.09% and process yield was 91.20 ± 6.40%. Microencapsulation process did not affect viral insecticidal activity and provided efficient protection against UVB radiation. Results demonstrated technological feasibility of spray drying process to be used in formulating a biopesticide based on NPV003.

Key words: Baculovirus, microencapsulation, spray drying, Eudragit® S100, photostability

INTRODUCTION

Spodoptera frugiperda (J.E. Smith 1797) (Lepidoptera: Noctuidae), the corn fall armyworm is a natural host for entomopathogenic viruses from the Baculoviridae family, principally the nucleopolyhedroviruses (NPVs) (Valicente and Costa,1995; Gomez et al. 2010). Some NPVs of *S. frugiperda* (S*f*MNPV) have been evaluated under field conditions, demonstrating high potential for pest control in corn crops (Williams et al. 1999; Moscardi 1999; Gómez at al. 2013). Evaluation under field conditions has produced inconsistent results, possibly due to the damaging effects of environmental conditions, such as solar radiation on viral particles (Burges 1998; Moscardi 1999; Caballero et al. 2001; Lacey and Arthurs 2005; Villamizar et al. 2009). Consequently, the formulations that encapsulate viral particles have been a preferred delivery system to minimize activity losses due to solar radiation (Tamez et al. 2002).

Microparticles can be obtained through several physical and chemical methods (Li et al. 2010), among which the spray drying process is an important one (Beck et al. 2004; Yu et al. 2007; Li et al. 2010). This process, performed in a fluid bed with top spray, includes the advantages of being fast, done in just one step, applicable to heat sensitive materials and easy to scale industrially (Beck et al. 2004; Yu et al. 2007; Jin and Custis 2011). In Colombia, three native isolations of

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S*f*MNPV codified as NPV003, NPV009 and NPV011 were physically, molecularly and biologically characterized demonstrating their high pathogenicity and virulence, from which the NPV003 isolate was selected due to its higher insecticidal activity and tolerance to ultraviolet radiation (Gómez et al. 2010). Considering the promising photo-stabilization results obtained in a previous study in which the virus was microencapsulated with Eudragit® S100 by solvent evaporation method (Villamizar et al. 2010), the aim of the present work was to improve on this formulation by using spray drying in order to solve such problems as low process efficiency (53.43%), bigger microparticle size and use of organic solvents, such as acetone and n-hexane.

MATERIAL AND METHODS

Materials

Eudragit® S100 (Röhm GmbH &Co.) was kindly donated by *Almapal* (Colombia); magnesium silicate mesh 325 pharmaceutical quality (Moisture 0.415%) and ethanol (96%) were purchased from *CIACOMEQ S.A.S* (Bogotá, Colombia); anhydrous dibasic sodium phosphate from *Sigma* (St. Louis, USA) and citric acid from *Merck* (Darmstadt, Germany**)**.

Insect rearing and virus

Insects were obtained from a colony of *S. frugiperda* established with larvae collected in corn fields in Espinal (Tolima Department), Colombia. Insects were kept in a room at 26 ± 2°C and 60% relative humidity with a 12 h photo-period. Larvae were kept individually in half ounce plastic containers with a fragment of artificial diet (Gómez et al. 2010). Virus NPV003 (also codified as SfCOL) was isolated from a *S. frugiperda* larvae collected from the pasture in Monteria, (Cordoba Department) Colombia in 2010.Viral occlusion bodies (OBs) of isolate NPV003 were produced by inoculating third instar *S. frugiperda* larvae with viral suspension at a concentration of 1 x 10^7 OBs/mL, using droplet feeding method (Hughes and Wood, 1981). Inoculated larvae were incubated at 26 ± 2°C and 60% relative humidity and fed with artificial diet until dying due to the infection. Dead larvae were collected, ground in distilled sterile water and homogenized. Mixture was filtered through a cloth layer to remove insect tissue and viral concentration was determined in a Neubauer counting chamber.

Polymer solution

Corresponding quantity of the polymer according to treatment (Table 1) was added to 70 mL of pH 8.0 phosphate buffer solution (972.5 mL of Na_2HPO_4 0.2 M and 27.5 mL of citric acid 0.1 M) with continuous agitation at high rpm. Then, 30 mL ethanol (96%) was added with continuous agitation until a clear solution was obtained.

Selection of optimal conditions for microencapsulation by spray drying

Microparticles were made with mixture of polymer solution and nucleus by spray drying in a fluidized bed with top spray (Glatt GmbH D–01277, Germany), using a 1.0 mm nozzle to adjust the air-flow. Inlet temperature was 80ºC, flow speed was 4.12 mL/minute and flap pressure was 25ºC in sprayer system. For this experiment, magnesium silicate was used as nucleus to simulate the viral OBs, considering similar particle size and high cost of virus production. Evaluated factors were chamber pressure, core concentration and polymer concentration in a factorial design with three factors and three levels for a total of 27 treatments (Table 1), each one carried out three times. Response variables were mean diameter of microparticles (d) and process yield. Factor influence was established with Statgraphics 8.1 software (v. 5.1, Manugistics Inc., Rockville, Maryland) by means of a response surface analysis and Pareto diagrams. A mathematical prediction model was generated and optimal process conditions were selected to proceed with virus microencapsulation.

Microparticle size and morphology

Shape and size of microparticles were studied with an optical microscope (NIKON ECLIPSE 300). Diameters of 150 particles from three samples of each treatment were determined with an image analyzer (Nis-Elements BR3.2). Microparticles morphology was determined by microphotographs taken with a scanning electron microscope (SEM)(Philips XL 20), following methodology described by Villamizar et al. (2010).

Yield

To estimate process yield, the material recovered from each batch of each treatment was weighed

and using this result and the weight of the used materials, the following formula was applied (Villamizar et al. 2010):

$$\text{Yield (\%)} = \frac{Wproduct}{(Wpolymer+Wnucleus)} x\ 100 \quad (1)$$

Where $W_{product}$ is the weight of the microparticles obtained in a production batch, $W_{polymer}$ is the weight of the polymer used in a production batch and $W_{nucleus}$ is the weight of the nucleus (active ingredient) used in a production batch.

Virus microencapsulation by spray drying
Active ingredient
A sample of 20 g of infected larvae was homogenized with 260 mL of distilled sterile water using an Ultraturrax IKA 40 (Jankel and Kunkel, Germany) disperser at 10,000 *x*g for one minute. Mixture was filtered through a cloth layer to remove insect tissue and viral concentration was adjusted with water to 3 x 10^9 OBs/mL. To increase the active ingredient mass, magnesium silicate at 10% was added as a diluent to viral suspension and vigorously shaken then mixture was spray dried in a fluidized bed with top spray (Glatt GmbH D–01277, Germany) until final moisture content of 3.57 $\pm$ 0.41%. Process conditions were: internal pressure of 1 bar; flow speed of 6.18 mL/minute; inlet temperature of 92ºC and flap pressure expressed as a 25º angle of aperture at process initiation and 35º at its conclusion. Dry virus in powder was collected in drying chamber and used as an active ingredient or nucleus in microencapsulated formulation.

Spray drying
With optimal conditions generated by a mathematical prediction model, dry virus microencapsulation was performed. Optimal values determined were: 6% of polymer concentration (Eudragit®S100), 1.35% of nucleus concentration (dry virus), and internal chamber pressure of 2.23 bars. Microencapsulation of viral isolation NPV003 was performed manufacturing three product batches. Other process conditions were: inlet temperature of 80ºC, flap pressure of 25º and 1.0 mm nozzle to adjust the air-flow. Only product deposited in collector of spray drying equipment was considered to be final product. Three samples from each batch were taken to determine microparticle morphology and diameter, using fore mentioned methods. Moisture content (%), viral concentration, encapsulation efficiency, residual content of solvents, biological activity and photostability were also studied.

Moisture Content (%)
A sample of 0.5 g was dried at 100ºC in a humidity analyzer (KERN MLS 50 – 3) until reaching constant weight. Results were expressed as a percentage and determination was carried out three times for each product batch (AOAC 2007).

Concentration of microparticles and encapsulation efficiency
Samples of 100 mg of each product batch were added to 9.9 mL of pH 5.0 citrate buffer and mixed for 5 minutes to wash microcapsules and release non-encapsulated OBs, which were estimated in a Neubauer counting chamber and under a light microscope (Olympus CH3ORF100). The total OBs concentration, in the product, was determined by mixing the samples of 100 mg of each batch with 9.9 mL of pH 8.0 phosphate buffer to promote polymer dissolution for releasing encapsulated OBs and counting in a Neubauer chamber and under a light microscope (Olympus CH3ORF100). Experimental concentration of microencapsulated OBs was determined by estimating the difference between total OB concentration after dissolution at pH 8.0 and non-encapsulated OBs released by washing microparticles at pH 5.0.
Then, encapsulation efficiency was calculated by using the following formula (Villamizar et al. 2010):

$$Encapsulation\ efficiency\ (\%) = \frac{Wr\ x\ 100}{Wt} \quad (2)$$

Where *Wr* is the experimental concentration of microencapsulated OBs and *Wt* is the theoretical content of the virus in the microparticles calculated using weight and concentration of polymer and active ingredient used for product preparation.
These characteristics were evaluated three times for each batch.

Biological activity
Bioassay was performed using the droplet feeding method (Hughes and Wood 1981). Suspensions were adjusted to five concentrations from 2x10^3 to 2x10^7OBs/mL for microencapsulated virus, and from 2x10^4 to 2x10^8OBs/mL for non-encapsulated virus (active ingredient). Neonates were starved at 25°C for 12h and then larvae were allowed to drink from aqueous suspension

prepared by mixing equal volumes of 10% (w/v) sucrose solution and 1% (w/v) food colorant E132 (Indigotine) with each viral treatment. Larvae that ingested suspension were transferred to individual recipients with artificial diet. Experiment was performed with 30 larvae per virus treatment and 30 larvae as negative control without any treatment. Larvae mortality was determined seven days after inoculation. Experimental design was completely random with factorial arrangement and three repetitions for each treatment. Mortality results underwent Probit analysis (Finney, 1952), using Biostat 2007 software to determine lethal concentrations.

Photostability of microencapsulated virus

Suspensions of microencapsulated and non-encapsulated virus (active ingredient) were prepared in distilled water and adjusted to a concentration of $2x10^7$OBs/mL. Samples of 200 µL of each suspension were placed in five continuous wells (column) of 96 well flat bottom plate, which was irradiated (302 nm) with a monochromatic UVB lamp at 302 nm and 8 watts (3UV™ lamp, UVP, Upland,CA) located at a distance of 10 cm, for 2, 4 and 6 h. Before beginning the irradiation, the first column of microplate wells was covered with aluminum foil (one well from each concentration), these treatments corresponded to non-irradiated or 0 hours of exposure. Every two hours until completing 6 h, the following column of wells was covered; thus, each column represented a different irradiation time. Neonatal larvae were starved at 25°C for 12 h and then larvae were allowed to drink from aqueous suspensions prepared by mixing equal volumes of a 10% (w/v) sucrose solution and 1% (w/v) food colorant E132 (Indigotine) with each viral treatment according to previously described method. To determine the efficacy, the Schneider – Orelli formula (Zar 1999) was used.

$$Efficacy\,(\%) = \frac{(A-B)}{(100-B)}\,x\,100 \qquad (3)$$

Where A is larva mortality obtained in treatment and B is mortality in negative control treatment.

Data normality and homogeneity were estimated using the Shapiro-Wilk (95%) and Barlett (95%) tests respectively. Then, differences between treatments were detected by LSD (95%) test using SAS (v9.1) Software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Optimal conditions for microencapsulation by spray drying

Microparticles with variable size and shape were obtained in all the evaluated treatments, with diameters ranging from 10.42 to 22.20 µm (Table 1). These values were similar to those obtained in microencapsulation by spray drying of *Beauveria brongniartii* conidium with skin milk, Kollidon and polyvinylpirrolidone (PVP K90), in which microparticles showed sizes between 3 and 20 µm (Horaczek and Viernstein 2004), as well as in the study in which *Bifidobacterium* BB-12 was microencapsulated with skin milk and product sizes measured from 14.45 to 18.78 µm (Fritzen-Freire et al. 2012).

To ensure that microparticles obtained through top spraying process could be ingested by neonatal *S. frugiperda* larvae to produce insecticide action, microparticles must be smaller than mouth openings of these larvae, which is approximately 70 µm (Villamizar et al. 2010). Keeping this in mind, and considering a diameter superior to OB sizes, which were approximately 2.0 ± 0.5 µm (Gómez et al. 2010), to guarantee viral microencapsulation, an adequate size range was selected between 10 and 40 µm and used for analyzing the effect of all the factors on particle size frequency.

The Figure 1A illustrates response surface for size frequency between 10 and 40 µm as a function of pressure and polymer concentration. It showed that response was maximized when polymer concentration tended toward level 0 (5.0%) and pressure did not affect the frequency of these particle sizes. When size particle frequency between 10 and 40 µm was correlated as a function of nucleus concentration and pressure (Fig. 1B), none of the factors had an effect on response variable. Nucleus and polymer concentrations (Fig. 1C) generated maximum response when polymer concentration fluctuated between levels 0 and +1 (5.0% and 7.5%, respectively) and nucleus concentration showed no effect.

Table 1 - Particle size and yield of spray dried microparticles prepared with Eudragit® S100 as a polymer and magnesium silicate as the nuclear agent using varied conditions.

Treatment	IP (bars)	Polymer (%w/v)	Nucleus (%w/v)	ITC (ºC)	d (µm)	Yield (%)
1	1	2.5	1.0	26.7-48.3	14.77	24.45
2	1	2.5	1.5	18.6-35.6	10.42	30.90
3	1	2.5	2.0	36.1-54.2	12.60	29.98
4	2	2.5	1.0	34.2-50.5	10.84	35.78
5	2	2.5	1.5	15.6-24.0	16.36	51.62
6	2	2.5	2.0	13.9-29.6	15.59	60.72
7	3	2.5	1.0	11.7-25.3	14.00	65.69
8	3	2.5	1.5	13.5-51.7	13.70	54.83
9	3	2.5	2.0	16.8-52.4	16.87	26.47
10	1	5.0	1.0	36.8-52.9	20.50	60.40
11	1	5.0	1.5	34.3-53.1	20.74	75.17
12	1	5.0	2.0	29.8-52.6	20.72	62.32
13	2	5.0	1.0	24.0-47.2	20.26	75.36
14	2	5.0	1.5	32.9- 50.0	19.55	92.46
15	2	5.0	2.0	26.7-47.7	22.20	57.30
16	3	5.0	1.0	24.6- 49.7	19.51	80.81
17	3	5.0	1.5	10.5- 47.7	19.62	78.61
18	3	5.0	2.0	30.6-47.7	19.11	60.86
19	1	7.5	1.0	35.5-48.3	19.28	88.06
20	1	7.5	1.5	35.6- 50.0	18.27	63.08
21	1	7.5	2.0	25.0- 52.7	21.22	62.64
22	2	7.5	1.0	41.5- 50.8	20.52	56.95
23	2	7.5	1.5	28.0- 47.3	18.95	86.51
24	2	7.5	2.0	20.4- 49.2	20.97	86.44
25	3	7.5	1.0	34.0- 53.7	18.99	73.69
26	3	7.5	1.5	26.7- 49.8	20.59	80.60
27	3	7.5	2.0	19.2- 50.7	18.53	49.32

[IP]: internal pressure, [ITC]: internal temperature Chamber, [d]: Average diameter of MPs.

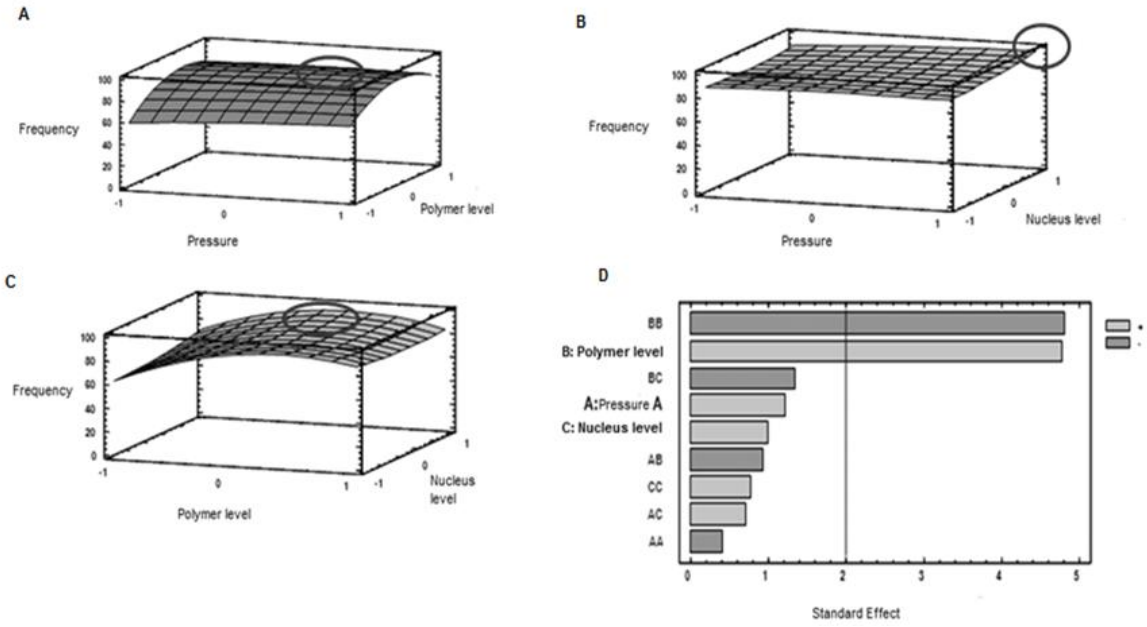

Figure 1A- Response surface of the frequency of size between 10 – 40 µm as a function of the pressure and polymer level; **B**: Response surface of the frequency of size between 10 – 40 µm as a function of the pressure and nucleus level; C: Response surface of the frequency of size between 10 – 40 µm as a function of polymer level and nucleus level for microparticles of Eudragit® S100; **D**: Pareto diagram.

Pareto diagram (Fig.1D) showed that polymer-polymer interaction and polymer concentration (B) were again the only factors that had a significant effect on particle size; thus leading to the conclusion that when polymer concentration was increased, the percentage of desired size microparticles was also increased. In some treatments, independent spherical particles were observed (results not shown), but in other treatments, big aggregates were produced, possibly due to coalescence of drops during the drying process. Some microparticles showed cavities or holes that could be attributed to the contraction generated by particle formation during the drying process due to rapid solvent evaporation (Fritzen-Freire et al. 2012).

In general, yield for the 27 treatments fluctuated between 24.45 and 92.46% (Table 1), a wide range also found by other authors who reported values between 72 and 76% (Beck et al. 2004); 21.60 and 62.30% (Tajber et al. 2009) and 50 and 70% (Li et al. 2010). These authors found that polymer concentration was the highest effect factor on yield, followed by polymer-nucleus-pressure interactions and pressure factor (results not shown). Later, a multi-criteria analysis generated a mathematical model to predict the optimum factor values so that maximum response for particle sizes between 10 and 40 µm and yield could be obtained. Predicted optimum parameters were pressure of 2.23 bars, polymer concentration of 6% and core concentration of 1.35%; and predicted responses were yield of 87.26 and 91.38% of particle with size between 10 and 40 µm.

Virus microencapsulation by spray drying

Dried virus (nucleus) had a concentration of 7.17 x 10^9 OBs/g, moisture content of 3.57 $\pm$ 0.41% and microbial contamination of 4.73 x 10^7 UFC/g, which were adequate characteristics for use as an active ingredient in a viral biopesticide with optimal conditions for the microencapsulation process predicted by the mathematical model. In this sense, dry virus was microencapsulated by using predicted optimum parameters, and microparticles presented the following characteristics.

Particle size and morphology

Size of microparticles of three production batches oscillated between 17.64 and 19.47 µm, with mean size of 18.84 $\pm$ 8.54 µm. Variation coefficient between the batches was lower than 10% (5.49%), suggesting high repeatability among batches. Mean microparticles diameter was not significantly different from the practical value previously determined for these treatments by using magnesium silicate as nucleus (23.85 $\pm$ 3.77 µm), confirming process repeatability. Analysis of the particle size frequency showed that 91% of microparticles presented desired size between 10 and 40 µm, a percentage, which did not differ from the theoretical value estimated under mathematical model (91.38%).

Spherical and irregularly shaped microparticles were obtained (results not shown). Aggregation of the microparticles was observed, a phenomenon possibly due to drop(s) coalescence during drying process. Clumping results from the formation of a liquid bridge between wet particles and occurs if evaporation capacity of the fluidized bed is low (Ronsse et al. 2009) or when ethanol is used as solvent, which has been shown to favor aggregate formation (Yoo et al. 2011). Different microparticles shapes suggest heterogeneous distribution of their components (Burki et al. 2011). The observed tendency of microparticles to wrinkle and form folds and cavities could be a consequence of contraction force and viscosity increase during the drop(s) drying process (Foster and Laetherman, 1995 in: Raffin et al. 2006). Moreover, hollow sphere morphology suggests a quick drying process with high temperature, close to or above solvent boiling point, which produces vapor inside the particle generating an empty core (Dobry et al. 2009).

Moisture content (%)

Moisture content for the three batches fluctuated between 9.4 and 11.06% with average value of 10.38 $\pm$ 0.87%. Variation coefficient among the batches was lower than 10% (8.38%), indicating high process repeatability. However, this moisture content was higher than recommended for this type of bio-products, whose humidity should be kept lower than 5% in order to maintain microbial stability during the storage (Jones and Burges 1998). Excessive moisture may favor proliferation of bacteria, fungi and other contaminating microorganisms (Caballero et al. 2001), and may also cause oxidation of components in the formulation and formation of free radicals, which can inactivate the virus (Jones and Burges 1998).

Yield

Process yield was 91.2 ± 6.4%, a value, which did not differ from that estimated by the mathematical model (87.26%). Yield showed an inverse relation with product moisture content, probably due to high material losses as a consequence of humid material deposit on internal equipment surfaces. A similar phenomenon was described by Tabjer et al. (2009), who obtained higher process yields (51.5 and 62.3%) during microencapsulation of *budesonide/formoterol fumarate* by spray drying, when moisture content was minimum and oscillated between 0.6 and 1.5%.

Concentration of microparticles and encapsulation efficiency

Theoretical microparticles concentration was estimated at 1.03 x 10^9 OBs/g based on viral concentration of active ingredient (5.46 x 10^9 OBs/g) and used quantities of nucleus and polymer. Mean experimental concentration of three batches was 8.73 x 10^8 OBs/g, and encapsulation process efficiency was estimated at 84.61 ± 13.09%, indicating that the majority of viral particles were microencapsulated. Developed process improved microencapsulation efficiency by more than 30% in comparison with microencapsulation of similar virus with Eudragit® S100 by solvent evaporation method, when efficiency was 53.43% (Villamizar et al. 2010).

High encapsulation efficiency can be obtained when microparticle solidification occurs very fast, due to factors such as low polymer solubility in organic solvent, high solubility of organic solvent in water, high polymer concentration, low (weak) relationship between disperse phase and continuous phase and fast solvent removal (Yeo and Park 2004). Consequently, high efficiency with developed process could be related to the use of ethanol as organic solvent, which presented high solubility in water, allowing for high mass transfer between the dispersed phase and continuous phase and favoring polymer precipitation (Jyothi et al. 2010).

Biological activity

Lethal concentration obtained for microencapsulated virus was 1.3 x 10^4 OBs/mL for batch 1; 3.1 x 10^4 OBs/mL for batch 2 and 3.1 x 10^4 OBs/mL for batch 3, with mean value of 2.5 x 10^4 OBs/mL. Fiducial limits (95%) obtained for LC_{50} of each batch overlapped, indicating no significant differences between the pathogenicity of the three products and suggesting high repeatability between the batches. LC_{50} of microencapsulated virus was lower than that obtained for nucleus or non-encapsulated virus (1.2 x 10^6 OBs/mL) and value reported by Gómez et al. (2010) for liquid suspension of pure virus (Table 2). However, when fiducial limits (95%) were compared, no significant differences were found between a treatment, which suggested same pathogenicity. This behavior led to the conclusion that the developed microencapsulation process did not affect insecticidal activity of viral occlusion bodies, as reported by Behle et al. (2003) during the encapsulation of *Anagrapha falcifera* nucleopolyhedrovirus with lignin by using spray drying, where insecticidal activity was not affected due to encapsulation process.

Table 2 - Mean lethal concentrations (LC_{50}) of microencapsulated, dried and unformulated nucleopolyhedrovirus NPV003 estimated over *Spodoptera frugiperda* neonates.

Treatment	LC_{50} (OBs/mL)	Fiducial Limits (OBs/mL) 95%		P	χ^2
		Lower	Upper		
Microencapsulated virus	2.5 x 10^4	1.7 x 10^3	5.9 x 10^5	0.70	1.42
Dried virus	1.2 x 10^6	2.1 x 10^5	3.1 x 10^6	0.30	3.64
Unformulated virus (Gómez et al. 2010)	2.3 x 10^5	5.4 x 10^4	4.7 x 10^6	0.25	4.72

Probit regressions were fitted using the Polo Plus program (LeOra software, 1987, Petaluma, CA, USA).

Photostability of microencapsulated virus

Initial efficacy (non-irradiated) of dried virus was 89.74% and after 6 h of irradiation, insecticidal activity was reduced to 20.51%. Microencapsulated virus presented initial efficacy of 96.18 ± 4.57%, a value that was reduced to 83.81 ± 5.24% after 6 h of irradiation (Fig. 2). This deleterious effect of UVB light was related to absorption of radiation by many biological molecules, including macromolecules such as DNA, causing direct lesions due to photoproduct formations such as pyrimidine dimers (appearance of covalent bonds between adjacent pyrimidimic bases: cytosine-cytosine or cytosine-thiamine),

pyrimidine hydrates and overlaps between DNA and proteins (Diffey 1991 en: Villamizar et al. 2009).

Prior to irradiation, efficacies were not significantly different among the three batches of microencapsulated and non-encapsulated virus ($F=2.03$, $df=3$; $p= 0.1879$) (Fig. 2), but after 2 h of UV exposure, non-encapsulated virus showed significantly lower activity ($F=21.12$, $df=3$; $p= 0.0004$). This behavior was also observed after 4 h ($F=31.89$, $df=3$; $p< 0.0001$) and 6 h ($F=22.6$, $df=3$; $p= 0.0003$) of irradiation of the non-encapsulated virus with inactivation of 69 and 77%, respectively. This result indicated that developed formulation was able to protect the viral OBs from the deleterious effect of ultraviolet radiation, which was possibly related to microcapsule coat. This acted as a physical barrier blocking the radiation due both to the polymer (Eudragit®S100) used and to the magnesium silicate added during the active ingredient elaboration.

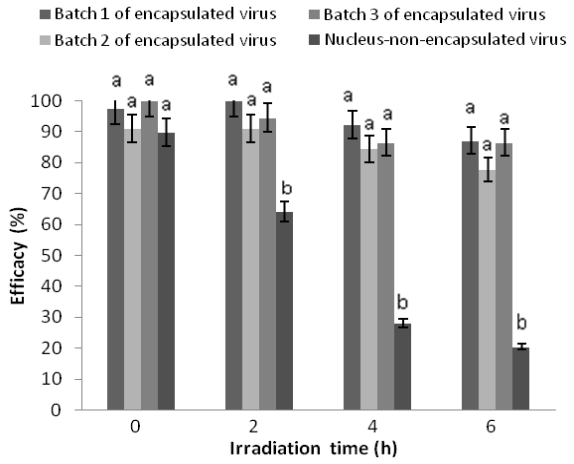

Figure 2 - Effect of the UVB radiation on the efficacy of *Spodoptera frugiperda* nucleopolyhedrovirus NPV003-Eudragit®S100-spray-dried microparticles. Treatments with the same letter was not significantly different according LSD (95%), applied independent for every time of irradiation.

Inactivation of microencapsulated virus in the present study was 8.67% after 4 h of exposure to UVB light (302 nm); similar results were obtained by Villamizar et al. (2010), who microencapsulated another isolation of this virus (NPV001) with Eudragit® S100 by solvent evaporation, which presented 13% inactivation following 4 h of irradiation with a ReptiGlo 8.0 lamp (33% UVA and 8% UVA), confirming the polymer protective effect against UV-light. Spray drying with other polymers as lignin has been used for baculovirus formulation improving OBs photostability as reported by Arthurs et al. (2006) who encapsulated *Cydia pomonella* (CpGV) granulovirus. Behle and Popham (2012) also encapsulated two *S. frugiperda* NPVs by spray drying with lignin, obtaining 35% inactivation of microencapsulated virus irradiated with simulated solar light, while non-formulated virus reduced its insecticidal activity by more than 94%.

CONCLUSION

This study revealed the technological feasibility for microencapsulating *S. frugiperda* NPV003 nucleopolyhedrovirus by spray drying using Eudragit® S100 as a polymer coating. The microencapsulation process under established conditions did not affect viral insecticidal activity and provided efficient protection from UVB radiation, presenting high potential for use in the formulation of other biological control agents.

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