

# Development and characterization of PLGA nanocapsules of grandisin isolated from *Virola surinamensis*: *in vitro* release and cytotoxicity studies

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**Abstract:** The most studied phyto constituent isolated from *Virola surinamensis* (Rol. ex Rottb.) Warb., Myristicaceae, is the tetrahydrofuran neolignan grandisin, which exhibits a series of biological activities, including trypanocidal, larvicidal and antitumoral. Due to its extremely low solubility, additional studies, including *in vivo* investigations are challenged by the difficulties in the development of an effective drug delivery system for grandisin. The encapsulation in polymeric nanoparticles is a very attractive alternative for overcoming some of these limitations. In this work, PLGA nanocapsules loaded with grandisin were developed in an attempt to optimize the efficacy of grandisin as an antitumoral drug, with high drug loading and efficiency, prolonged drug release and increased physical-chemical stability. Mean diameter of the nanocapsules was lower than 200 nm, with very low polydispersity. Encapsulation efficiency was above 90%. A sustained *in vitro* drug release was achieved for up to twenty days and cytotoxicity was markedly increased (IC<sub>50</sub> for grandisin-NC and grandisin were 0.005  $\mu$ M and 0.078  $\mu$ M, respectively), indicating that polymeric nanocapsules are a potential drug delivery system for grandisin allowing the preparation of formulations viable for further *in vivo* studies.

## Introduction

*Virola surinamensis* (Rol. exRottb.) Warb., Myristicaceae, popularly known as “ucuúba-branca” or “ucuúba-de-igapó”, has been used in folkmedicine in the treatment of erysipelas, colic and dyspepsia (Schultes & Holmstedt, 1971). Phytochemical investigations on these species revealed the presence of steroids, flavonoids, polyketide, tetrahydrofuran lignans and one aryltetralinneolignan. Currently, the most studied phytoconstituent isolated from *Virola surinamensis* is the tetrahydrofuran neolignan grandisin (GRAN). The tetrahydrofuran lignans isolated from adult leaves and woods showed high trypanocidal activity against the tripomastigote forms of *Trypanosoma cruzi*, the aetiologic agent of Chagas disease (Lopes et al., 1996; Lopes et al., 1998). Additionally, *in vitro* effectiveness of GRAN as larvicidal agent against *Aedes aegypti*

and *Chrysomyamegacephala* has been demonstrated (Nogueira et al., 2009; Cabral et al., 2009).

More recently, *in vivo* studies have demonstrated that GRAN has antinociceptive and anti-inflammatory properties (Carvalho et al., 2010). Also, our research group demonstrated its marked antitumoral activity against Ehrlich ascites tumor experimental model (Valadares et al., 2009) and an important antileukemia activity on K-562 cell line (Valadares et al., 2011).

GRAN, however, presents challenging properties regarding the development of dosage forms appropriate for further *in vivo* investigations. These undesirable properties include a practically null hydrosolubility and high lipophilicity, which has been considered a limiting factor to address pharmacological and toxicological investigations in animals. Therefore, investigations on the development of suitable formulations in order to improve GRAN



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pharmacokinetics, which is associated with the efficacy of antitumoral drugs, are necessary. Likewise, the increase of an apparent drug solubility and the control of drug release could also improve GRAN drug delivery.

The encapsulation of drugs in polymeric nanoparticles is a very attractive alternative for overcoming some of these limitations. Polymeric nanoparticles, such as poly(lactic-co-glycolic acid) (PLGA) nanoparticles, have been extensively used for the encapsulation of lipophilic drugs (Budhian et al., 2007). These nanoparticles are able to increase drug concentration in the formulation, maximizing the amount of drug in tumor cells. Moreover, due to the EPR effect (enhanced permeability and retention of solid tumors), more particles may be retained in the tumor, releasing the cytotoxic drug in a controlled manner (Souza et al., 2011).

Thus, the aim of this work was to develop GRAN-loaded PLGA nanocapsules using interfacial deposition of the preformed polymer (nanoprecipitation), in an attempt to optimize the efficacy of GRAN as an antitumoral drug, with high drug loading and efficiency, prolonged drug release and increased physical-chemical stability.

## Materials and Methods

### Materials

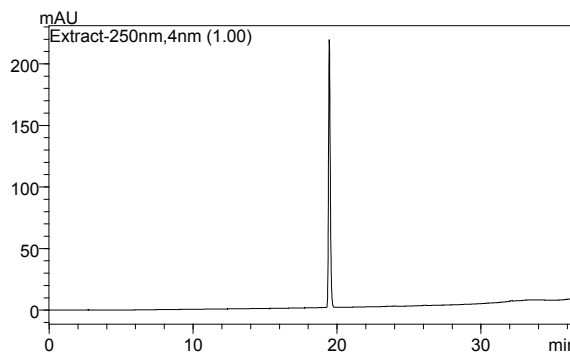
Poly(lactic-co-glycolic acid) (PLGA) and poloxamer (Pluronic F68® and Pluronic F127®) were from Sigma-Aldrich (USA), soy phosphatidylcholine 99% (PC) was from Lipoid Gmb (Germany). Ultrapure Milli-Q water was used throughout the study. All other chemicals were of analytical grade or higher.

### Grandisin

Grandisin (GRAN) (molecular weight: 432.2) was obtained from the leaves of *Viola surinamensis* (Rol. ex Rottb.) Warb., Myristicaceae, similarly to a previously reported method (Martins et al., 2000). The plant material was collected at Combu Island (01°30'10"S; 048°27'42"W), near Belém-PA, Brazil. A dry voucher sample (LOPES-037) has been deposited in the SPF-Herbário do Instituto de Biociências da Universidade de São Paulo.

A fraction (1.7 g) from crude EtOAc extract obtained *V. surinamensis* leaves was submitted to a column chromatography under vacuum (h:d: 150 x 100 mm) using hexane:EtOAc (4:1-4:1) yielding 22 fractions. A pooled fraction (16-20) was submitted to recrystallization in MeOH yielding 80mg of grandisin. The HPLC analysis using C-18 column [250x5 mm, 5µm particle, detector set at 250 nm; MeOH:H<sub>2</sub>O,

1:1(5')-1:0(30') 1 mL/min] indicated chromatographic purity >99% (Figure 1). The identity of the grandisin was confirmed by direct chromatographic comparison with authentic standard compound previously isolated and by <sup>1</sup>H NMR spectrum (Martins et al., 2000).



**Figure 1.** HPLC analysis of grandisin isolated from *Viola surinamensis*.

### Analytical procedure

The amount of GRAN in the nanocapsules was determined by a UV high-performance liquid chromatography method (HPLC), with Varian ProStar separation module equipped with a ProStar 310 UV detector, ProStar 240 solvent delivery pump and a ProStar 410 auto injector system (Varian Inc., USA). Separation was performed in a Lichrosphere 100 RP-18 (250 mm x 4.6 mm, 5 µm, Darmstadt, Germany, Merck) column. The mobile phase was H<sub>2</sub>O:ACN:MeOH (20:10:70 v/v/v) at a flow rate of 1 mL/min, injection volume of 50 µL, 25°C. Elution time for GRAN peak was 2.24 min and detection was carried out at 269 nm. A linear calibration curve ( $y=4.099x-3.683$ ;  $r=0.9999$ ) was obtained over the working concentration range of 50-150 µg/mL.

The amount of GRAN quantified in receptor solution (for the release studies) was determined with the following modifications to the previously described chromatographic method: the mobile phase was H<sub>2</sub>O:ACN:MeOH (20:30:50 v/v/v) at a flow rate of 1.8 mL/min, injection volume of 50 µL, 25°C. The same RP-18 column was used. Elution time for GRAN peak was 3.8 min and detection was also carried out at 269 nm. A linear calibration curve ( $y=6.319x-0.075$ ;  $r=0.9999$ ) was obtained over the working concentration range of 0.8-50 µg/mL.

### Preparation of GRAN nanocapsules (GRAN-NC)

Grandisin-loaded nanocapsules dispersions (GRAN-NC) were prepared by interfacial deposition of the preformed polymer (nanoprecipitation), based on the method proposed by Fessi (1989). Briefly, the

organic phase containing PLGA (150 to 200 mg), PC (150 mg), soybean oil (100 mg), methanol (5 mL), acetone (22 mL) and different amounts of GRAN (5, 8, 10 e 12 mg) was mixed at room temperature. The aqueous phase consisted of phosphate buffer pH 7.4 with 150 mg of Pluronic F68® or a mixture of Pluronic F68® and Pluronic F127® (1:1). The organic phase was slowly injected into the aqueous phase under magnetic stirring, with the aid of a 10 mL syringe attached to a needle with 0.3175 mm internal diameter. The nanoemulsion remained under magnetic stirring for 30 min at room temperature. Then, the organic solvents were removed by evaporation under reduced pressure (600 mmHg) at 40°C, in a rotary evaporator, to a final volume of 10 mL. Blank nanocapsules without GRAN (blank NC) were also obtained.

#### Physicochemical characterization of nanocapsules

Physical aspect, particle size and zeta potential

Nanocapsules dispersions were observed for their homogeneity, color, presence of residue or precipitates and phase separation. The mean diameter (nm) and polydispersity index (PDI) of GRAN-NC dispersions (diluted 1:200 with purified water) were measured by dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK) at 25°C. Zeta potential was measured by electrophoretical mobility and analyzed in folded capillary cells with the same instrument and experimental conditions.

#### Entrapment efficiency (EE%)

In order to isolate the entrapped GRAN from the free drug, 1 mL of recently prepared GRAN-NC dispersions were centrifuged (1620 x g) for 15 min. Precipitate containing free drug was dissolved with methanol and withdrawn for HPLC analysis (see section 2.3). All analyses were performed in triplicate. Total amount of drug incorporated in NC was determined by disrupting the nanocapsules with methanol followed by HPLC quantification. The entrapment efficiency (EE) and drug loading (DL), were determined using Equation 1 and 2:

$$EE(\% w, w) = \frac{\text{amount of GRAN in the formulation} - \text{amount of free drug}}{\text{amount of GRAN in the formulation}} \times 100 \quad (1)$$

$$DL(\% w, w) = \frac{\text{amount of GRAN in the formulation} - \text{amount of free drug}}{\text{weight of polymers}} \times 100 \quad (2)$$

#### Stability studies

GRAN-NC dispersions were stored at 4±2°C

for thirty days. Stability was evaluated by comparing the initial particle size, PDI (polydispersity index), pH and GRAN initial concentration with those obtained from samples withdrawn after 1, 5, 10 and 30 days of storage at 4°C.

#### In vitro release studies

Drug release was evaluated by a dialysis-diffusion method. One milliliter of GRAN-NC (with 1 mg/mL of GRAN) was introduced into a dialysis bag (regenerated cellulose membrane with a molecular weight cut-off of 12,000-15,000 Da). The release experiment was initiated by placing the dialysis bag in 10 mL of a recipient medium (phosphate buffer pH 7.4 with 5% sodium dodecyl sulfate (SDS), to maintain sink conditions) stirred at 100 rpm and 37°C. At predetermined intervals (3, 6, 9, 12, 24, 36, 48 h; 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 19, 21 days), aliquots (500 µL) of the recipient solution were withdrawn and replaced with fresh medium. Samples were analyzed for GRAN content by HPLC.

#### Cytotoxicity studies

Balb/c 3T3-A31 fibroblasts were cultured in Dullbecco's Modified Eagle's Medium (DMEM-D5648 Sigma Aldrich), supplemented with 10% fetal bovine serum (FBS-GIBCO), 100 IU/mL penicillin, and 100 µg/mL streptomycin, and routinely grown as a monolayer in 75 cm<sup>2</sup> tissue culture flasks (TPP) at 37±1°C, 90±10% humidity, 5.0±1.0% CO<sub>2</sub>/air. The cells were examined in a daily basis under a phase contrast microscope, and any changes in their morphology or adhesive properties were registered. For the experiment, cells were removed from the culture flasks using trypsinization (0.25% trypsin and 0.02% EDTA solution) when they exceeded 50% confluence but before reaching 80% confluence. A cell suspension containing 3x10<sup>4</sup> cells/mL was prepared on the day of plate seeding using culture medium supplemented with 10% FBS. The first row (blanks) of 96-well microliter plates were seeded with 100 µL routine culture medium and the remaining wells received 100 µL of a suspension containing 3x10<sup>4</sup> cells/mL (3x10<sup>3</sup> cells/well) and were incubated for 24±2 h (37±1°C; 90 ±10% humidity, 5.0±1.0% CO<sub>2</sub>/air) so that the cells could form a monolayer less than 50% confluent. This incubation period assured the cell recovery and adherence and progression to exponential growth phase. For neutral red uptake (NRU) assays, a Balb/c 3T3-A31 cell suspension containing 3 x 10<sup>4</sup> cells/well was seeded in 96-well plates and after 24 h, time required for the growing cells to attach to the bottom of the flask and for cell recovery, they were treated with eight different concentrations of freshly

prepared test GRAN or GRAN-NC in complete medium (six wells per concentration) and incubated for 48 h. The control wells (blanks) received complete culture medium supplemented with 10% FBS. After that, 250  $\mu$ L of neutral red (NR) medium were added to all wells, including the blanks, and incubated ( $37\pm 1^\circ\text{C}$ ,  $90\pm 10\%$  humidity,  $5.0\pm 1.0\%$   $\text{CO}_2/\text{air}$ ) for  $3\pm 0.1$  h. The cells were briefly observed between 2 and 3 h after incubation for NR crystal formation. After 3 h, the NR medium was removed and the cells were carefully rinsed with 250  $\mu$ L/well of pre-warmed PBS. The PBS was decanted from the plate and 100  $\mu$ L of NR desorb (50 EtOH: 1  $\text{CH}_3\text{CO}_2\text{H}$ : 49  $\text{H}_2\text{O}$ ) solution were added to all wells, including the blanks. The plates were rapidly shaken on a microplate shaker for 20 min to extract NR from the cells and to form a homogeneous solution. The absorption was measured 545 nm in a microtiter plate reader (spectrophotometer). The optical density (OD) was calculated as the difference between the absorbance at the test wavelength and the reference wavelength. For each concentration tested, wells containing all the reagents used but no cells served as reference blanks.

#### Statistical analysis

Data from the microtiter plate reader were transferred to a spreadsheet template Microsoft Office Excell 2007<sup>®</sup> for the calculation of cell viability, IC50 values by linear interpolation, and statistical analyses. Concentration-response curves were constructed and fitted in Microsoft Office Excell 2007<sup>®</sup> using parametric nonlinear regression. IC50 values were computed using the fitted Hill equation and presented as mean $\pm$ SD of at least two independent experiments. Each experiment was carried out in six repetitions (twelve values for each compound).

## Results and Discussion

### Nanocapsules preparation

Previously to the encapsulation of GRAN-

NC, blank nanocapsules were prepared with different concentrations of PLGA and surfactants. Table 1 shows mean diameter and polydispersity index (PDI) values obtained from the different nanocapsules formulation.

Nanoparticles obtained with 150 mg of Pluronic F68<sup>®</sup> and different concentrations of PLGA did not presented significant differences in mean diameter ( $p>0.05$ ). However, the increase in PLGA concentration also increased PDI values ( $p<0.05$ ), probably due to aggregation of the free polymer chains that did not form nanocapsules. Nanoparticles obtained with the mixture of poloxamers and lower amount of PLGA (150 mg) had a smaller diameter and a narrower size distribution ( $p<0.05$ ). Since surfactants play a main role in the formation of the nanodispersion, the combination of surfactants with different molecular weights (8,400 and 12,600 for Pluronic F68<sup>®</sup> and Pluronic F127<sup>®</sup>, respectively) favored the formation of smaller nanoparticles (Liu et al., 2011). Nanocapsules obtained with the mixture of poloxamers were then selected for GRAN incorporation.

**Table 1.** Mean diameter and PDI values of nanocapsules prepared with different amount of PLGA and poloxamers.

PLGA (mg)	Pluronic F68 <sup>®</sup> : Pluronic F127 <sup>®</sup> (mg)	Mean diameter (nm) <sup>a</sup>	PDI <sup>a</sup>
200	150:0	218.0 $\pm$ 18.4	0.547 $\pm$ 0.002
165	150:0	206.0 $\pm$ 12.7	0.454 $\pm$ 0.011
150	150:0	212.0 $\pm$ 11.3	0.379 $\pm$ 0.009
150	75:75	188.4 $\pm$ 3.50 <sup>b</sup>	0.139 $\pm$ 0.023 <sup>b</sup>

<sup>a</sup>Mean $\pm$ SD of at least three measurements; <sup>b</sup>Nanocapsules were smaller and less polydisperse than the other formulations ( $p<0.05$ ).

### Physicochemical characterization of GRAN loaded nanocapsules

GRAN-NC dispersions were successfully obtained by interfacial deposition of the preformed polymer (nanoprecipitation). In an attempt to achieve high encapsulation efficiencies (EE) and drug loading (DL), different amounts of GRAN were added to the

**Table 2.** Mean diameter, polydispersity index (PDI), entrapment efficiency (EE), drug loading (DL) and zeta potential of nanocapsules with and without GRAN\*.

Amount of GRAN added (mg/mL)	Mean diameter (nm) <sup>a</sup>	PDI <sup>a</sup>	EE (% w/w) <sup>a</sup>	DL (% w/w) <sup>a</sup>	Zetapotential (mV) <sup>a</sup>
-	163.2 $\pm$ 1.56	0.140 $\pm$ 0.006	-	-	-5.50 $\pm$ 0.85
0.5	174.9 $\pm$ 1.98	0.115 $\pm$ 0.008	100.22 $\pm$ 0.84	3.34 $\pm$ 0.02	-6.64 $\pm$ 0.55
0.8	170.1 $\pm$ 1.27	0.135 $\pm$ 0.019	100.00 $\pm$ 0.35	5.33 $\pm$ 0.02	-8.60 $\pm$ 0.29
1.0	169.5 $\pm$ 0.28	0.141 $\pm$ 0.004	98.20 $\pm$ 0.65	6.54 $\pm$ 0.04	-10.82 $\pm$ 0.43 <sup>b</sup>
1.2	173.2 $\pm$ 0.35	0.121 $\pm$ 0.041	90.45 $\pm$ 0.38	7.23 $\pm$ 0.03	-14.06 $\pm$ 2.32 <sup>b</sup>

\*Formulations obtained with 150 mg of PLGA and 150 mg of a mixture of Pluronic F68<sup>®</sup> and Pluronic F127<sup>®</sup> (1:1); <sup>a</sup>Mean $\pm$ SD of at least threemeasurements; <sup>b</sup>GRAN-NC presented higher zeta potential (in *modulus*) compared to the GRAN-NC with 0.5 mg/mL ( $p<0.05$ ).



**Table 3.** Mean diameter, Pdl, entrapment efficiency and pH of GRAN-NC dispersions during stability evaluation at 4°C.

Time (days)	Mean diameter (nm) <sup>a</sup>	Pdl <sup>a</sup>	Entrapment efficiency (% w/w) <sup>a</sup>	pH <sup>a</sup>
0	161.5±0.35	0.123±0.01	99.99±0.24	7.24±0.001
1	159.4±0.78	0.115±0.01	100.37±0.53	7.33±0.006
5	161.1±0.42	0.150±0.02	96.30±0.28 <sup>c</sup>	7.26±0.012
10	160.5±0.85	0.120±0.04	96.13±0.04 <sup>c</sup>	7.25±0.006
30	257.5±1.20 <sup>b</sup>	0.270±0.01 <sup>b</sup>	93.55±0.50 <sup>c</sup>	7.10±0.015 <sup>d</sup>

<sup>a</sup>Mean±SD of at least threemeasurements, <sup>b</sup>Mean diameter and Pdl increased in thirtydays ( $p<0.05$ ); <sup>c</sup>Entrapment efficiency decreased compared to the initial concentration (day 0 and 1) ( $p<0.05$ ); <sup>d</sup>pH decreases compared to the initial value (day 0) ( $p<0.05$ ).

PLGA nanocapsules formulations (5, 8, 10 e 12 mg), resulting in concentrations of 0.5, 0.8, 1.0 and 1.2 mg/mL of GRAN. Table 2 demonstrates physical-chemical characteristics of the nanocapsules.

Higher amounts of GRAN in the formulation did not alter significantly the size and Pdl of nanocapsules ( $p>0.05$ ). GRAN loading in the nanocapsules ranged from 3.3 to 7.20% (w/w). However, the addition of more than 1.0 mg of GRAN significantly increased the amount of non-encapsulated drug, which reduced entrapment efficiency values to about 90%, indicating that a maximum efficiency had been reached.

Also, zeta potential data demonstrated that the increase of the amount of GRAN in the formulation increased (*in modulus*) zeta potential values ( $p<0.05$ ), which was probably related to the surface-associated drug in the nanocapsules, as previously reported in the literature (Souza et al., 2011). The following studies were performed with the formulation containing 1 mg/mL of GRAN, since this formulation showed the highest EE, a small and uniform size, and a high DL value (Table 2).

### Stability studies

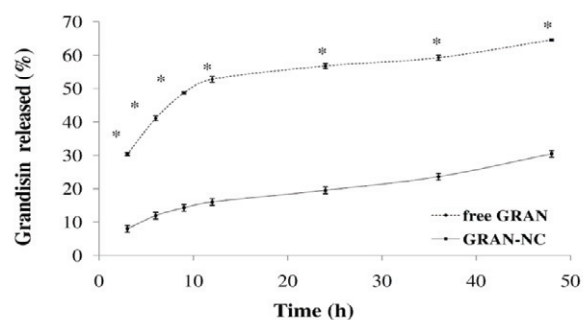
Stability studies of GRAN-NC were performed in order to assess the influence of nanoparticles ingredients on the stability of the colloidal suspensions. For this purpose, mean diameter, Pdl, entrapment efficiency (EE) and pH of GRAN-NC dispersions were determined after different storage times (1, 7, 15, 30, 90 days) at 4°C for thirtydays. Stability data are shown in Table 3.

After thirtydays, GRAN-NC dispersions were apparently homogeneous, without any visible precipitates, phase separation or flocculation. Until tendays, GRAN-NC did not exhibit alterations in particle size and Pdl ( $p<0.05$ ). In 30 days, slightly increase in size and Pdl could be observed without any loss in the homogeneity of the formulation. Entrapment efficiency and pH decreased with time. The decrease in GRAN-NC entrapment efficiency could be an indicator of PLGA degradation during the experiment. It has

been discussed that polymer degradation usually leads to a drop in pH value (Mathias et al., 2006), which was observed in these formulations. Thus, it seems that a small amount of GRAN was released during storage due to polymer degradation in the aqueous environment.

### In vitro release studies

Previous studies have demonstrated that the slow and prolonged release of antineoplastic drugs could modify drug cytotoxicity due to modifications on the exposure profile of the cancer cells to drugs (Souza et al., 2011). As GRAN has a great potential as an antineoplastic drug (Valadares et al., 2009), the release profile of GRAN from nanocapsules was determined and the results are shown in Figure 1. Diffusion of free GRAN through the cellulose membrane was faster than from the drug encapsulated in nanocapsules. Approximately 65% of GRAN is released from a micellar dispersion in 48 h, while only 30% of GRAN was released from nanocapsules.

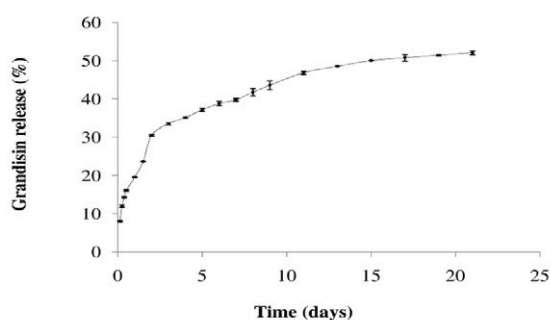


**Figure 2.** Diffusion of free GRAN and GRAN-NC through the cellulose membrane in 48 h of experiment. \* $p<0.05$ .

Drug release experiments of GRAN-NC were also performed for 21 days to determine the drug release profile for a longer period of time. Figure 2 demonstrates that 52% of GRAN is released from nanocapsules in 21 days.

Thus, in the first 48 h, approximately 31% of the drug was released from nanocapsules, and only 21% of the drug was additionally released in the

following nine ten days. This two-stage release may be related to the design of the nanocapsules, since they present a core-shell type structure, in which the drug could be associated to the surface of the nanocapsules and/or dissolved into the core (Melo et al., 2011). As such, in this experiment, within the first 48 h, GRAN surface-associated is released and then, as GRAN is a highly lipophilic drug, the remaining drug dissolved in the oily nucleus is released from nanocapsules in a sustained manner. In summary, GRAN-NC seems to be a potential carrier for GRAN delivery, whereas high drug loading and sustained drug delivery was achieved.



**Figure 3.** Release of grandisin from PLGA nanocapsules.

#### *In vitro* cytotoxicity studies

Studies have shown that nanoencapsulation could increase the drug uptake into cells, which, in turn, increases drug cytotoxicity. Also, nanoencapsulation of cytotoxic drugs has been considered a great strategy to overcome multidrug resistance, *i.e.* the efflux of free drug caused by glycoproteins overexpressed in tumor cell membranes (Wong et al., 2007).

In an attempt to verify if PLGA nanocapsules could increase GRAN cytotoxicity, *in vitro* cytotoxicity studies were performed with NRU assay and 3T3 cell lines. Different concentrations of GRAN-NC and free GRAN (2.31; 1.16; 0.58; 0.29; 0.14; 0.07; 0.04; 0.002 µg/mL) were added to the cell culture, which was incubated for 48 h. The IC<sub>50</sub> values for GRAN-NC and GRAN were 0.005 µM and 0.078 µM, respectively.

The decrease in IC<sub>50</sub> value for nanocapsules showed that the nanoencapsulation modified the cytotoxic profile of the GRAN on fibroblast cells, which might be related to the increase in drug cellular uptake from PLGA nanocapsules. It has been reported that while free drug enters the cells by passive diffusion, nanoparticles could be internalized by endocytosis (Zhao et al., 2011; Oh et al., 1999), and this mechanism of internalization could also occur in tumor cells (Zhao et al., 2011). In addition, studies reported that *in vivo* administration of nanoparticles may increase drug accumulation in solid tumors due to their irregular

vasculature (EPR effect) (Souza et al., 2011). Thus, GRAN nanoencapsulation has potential to be a more effective form to treat tumors, since it can be related to a higher cellular uptake and drug accumulation in the tumor tissue. Further experiments will be performed in different cell lines and *in vivo*, in order to study GRAN cellular uptake and tissue accumulation of nanocapsules.

#### Conclusion

In this work GRAN, isolated from *Viola surinamensis* (Rol. ex Rottb.) Warb., Myristicaceae was successfully loaded in a PLGA nanocapsule dispersion. Nanoencapsulation sustained GRAN release, provided chemical stability and altered its cytotoxicity. GRAN-NC seems to be a potential carrier for GRAN delivery for further *in vivo* studies.

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