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# Smart design of intratumoral thermosensitive β-lapachone hydrogels by Artificial Neural Networks

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ABSTRACT

This study presents Artificial Neural Networks (ANN) as a tool for designing injectable intratumoral formulations of the anticancer drug β-lapachone. This methodology permits insight into the interactions between variables and determines the design space of the formulation without the restrictions of an experimental design. An ANN model for two critical parameters of the formulations; the amount of solubilized drug and gel temperature was developed and validated. The model allowed an understanding of interactions between ingredients in the formulation and the fundamental phenomena as the formation of polypseudorotaxanes to be detected and quantified.



## 1. Introduction

β-Lapachone (βLAP) is an anticancer agent displaying a potent therapeutic activity against cancer cells (Ferreira et al., 2009; Jeong et al., 2009). Recent studies have demonstrated that βLAP induces apoptosis through a reaction catalyzed by NQO1 (NAD(P)H: quinone oxidoreductase) (Park et al., 2005). The mechanism, independent of caspases, p53 status and cell cycle stage, could be a new strategy for the selective treatment of NQO1 on expressed tumors (Blanco et al., 2007), such as pancreatic (Ough et al., 2005), pulmonary (Bey et al., 2007), mammarian (Blanco et al., 2007) and prostatic (Dong et al., 2009).

Two βLAP characteristics hinder its clinical use; its poor solubility in water (0.04 mg/mL) (Nasongkla et al., 2003) and its non-specific distribution (Ough et al., 2005) could be circumvented by applying different technological approaches.

The formation of inclusion complexes with cyclodextrins (CDs), especially hydroxypropyl-βCD and a randomly methylated-βCD has been shown to be useful in greatly increasing βLAP water solubility (Cunha-Filho et al., 2007; Dong et al., 2009; Nasongkla et al., 2003; Wang et al., 2006).

On the other hand, one attractive alternative of avoiding antitumoral drugs non-specific distribution is its local administration (Dong et al., 2009). Such approach should assure precision in the local drug delivery with minimal systemic toxicity and a dramatic increase in tumor tissue concentrations compared to conventional systemic chemotherapy (Yang et al., 2009). Thus, different types of formulations have been proposed; emulsions (Karasulu et al., 2004; Takahashi et al., 1976), liposomes (French et al., 2010; Hwang et al., 2007), nanoparticles (Donghui et al., 2009; Park et al., 2010), polymer implants (Dong et al., 2009; Ranganath et al., 2010) and hydrogels (Gupta, 1990; Yang et al., 2009). Some of which have become commercially available as Gliadel® or IntraDose® (Al-Abd et al., 2010; Weinberg et al., 2007), but bearing the patient's comfort and cost reduction in mind, the research interest has recently shifted from implants to injectable formulations (Van Tomme et al., 2008).

Pluronic F127® (PF127) is one of the most important polymers in the development of thermosensitive injectable systems (Kabanov et al., 2002a). Its low toxicity after parenteral administration (Johnston and Miller, 1985) together with its rheological properties promoted, over the last few years, different studies on developing these kinds of formulations. Pluronic F127 can form siringable systems at room temperature that undergo sol–gel transitions at physiological temperature making a depot after injection, from which the drug is controlled released (Jeong et al., 1997). Although, its structure allows the formation of micelles that contribute to solubilizing lipophilic drugs, it has been demonstrated that its solubilization capacity is not always enough to assure the adequate pharmacological activity and other additives like cosolvents or surfactants should be included in the formulation (Yang et al., 2009). However, additives have also an effect on the critical characteristics of those systems, such as rheological properties or temperature sensitivity (Dreiss et al., 2009; Nogueiras-Nieto et al., 2009; Valero and Dreiss, 2010; Simões et al., 2012).

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On this basis, it is clear that the interactions between pluronic and cyclodextrins and βLAP and cyclodextrins should give ternary systems of which properties are not easily predictable. The development of a thermosensitive injectable formulation involves the understanding of interactions between all the components (Pluronic-drug-additives) and in the last term, the selection of a balanced composition to obtain an optimal formulation which often means a compromise solution.

Artificial Neural Networks (ANNs) can be considered a useful tool for modeling whose advantages over conventional statistical and mathematical techniques have been well established (Arulsudar et al., 2005; Landin et al., 2009). ANNs can be defined as biologically inspired computer programs, designed to simulate the way in which the human brain processes information (Takayama et al., 1999). These computational techniques allow, through experience, the establishment of relationships between the variables of a process (inputs) and its results (outputs). Complete and updated information on the applicability of this technique can be found in Colbourn and Rowe (2009).

In this study we intend to model the influence of the proportion of ingredients; polymer (pluronic) and solubilizing agents (cyclodextrin) in developing intratumoral thermosensitive βLAP systems using ANNs. Such a model will provide an insight into the interactions between the ingredients and will help us to obtain injectable formulations with enough dose of βLAP, which could be able to form a local depot after intratumoral injection with a controlled drug release for an extensive period of time.

## 2. Materials and methods

β-Lapachone (batch L503; 3,4-dihydro-2,2-dimethyl-2H-naphthol-[1,2-b]pyran-5,6-dione; $C_{15}H_{14}O_3$; MW 242.3) was produced by Laboratorio Farmaceûtico do Estado de Pernambuco, LAFEPE (Recife, Brazil) with a purity estimated by DSC and HPLC in 99.9%. Randomly methylated-β-cyclodextrin (RMβCD: degree of molar substitution 0.57) was donated by Roquette (Barcelona, Spain). Pluronic F127® (PF127, 12,600 Da) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Purified water was obtained using reverse osmosis (MilliQ®, Millipore, Barcelona, Spain).

### 2.1. Preparation of PF127, RMβCD or PF127-RMβCD dispersions

Systems containing PF127, RMβCD or combinations PF127-RMβCD were prepared at the concentrations indicated in Table 1. PF127 solutions were prepared using the cold method described by Kabanov et al. (2002b). Accurate amounts of RMβCD were dissolved in a small volume of distilled water and added to PF127 solutions.

### 2.2. β-LAP solubilization

An excess of βLAP was added to 3 g of the different systems and maintained under stirring at low temperature until equilibration. Filtered solutions (0.45 μm filter Millipore Corp., Billerica, USA) were accurately diluted with water/ethanol solution (1:1) and the solubilized βLAP was analyzed spectrophotometrically at 257 nm (Agilent 8453, Santa Clara, USA).

Solubility *Enhancement Factor* (EF) was calculated by dividing the βLAP solubilized in the system by βLAP solubility in water (38 μg/mL).

### 2.3. Rheological characterization

Rheological analysis of the systems was performed on a controlled stress rheometer (Rheolyst AR-1000N TA instruments, UK) equipped with a Peltier plate for temperature control using a cone-plate geometry (60 mm diameter with an angle of 1.58°, gap 50 μm). Ramps of temperature from 15 °C to 37 °C at 2 °C/min with an oscillatory stress of 0.1 Pa at 5 rad/s were carried out. Gel temperature ($T_{gel}$) was estimated from the cross point between the storage moduli ($G'$) and loss moduli ($G''$).

Additionally, frequency sweeps from 0.05 to 50 rad/s were carried out at 0.1 Pa at 15 °C and 37 °C.

### 2.4. In vitro drug release studies

The βLAP release profiles were determined in triplicate using horizontal diffusion cells (Crown glass Corp., Somerville, NJ, USA) at 37 °C. A dialysis membrane of a molecular weight cut-off of 7.0 kDa (Medicell International Ltd., UK) and a diffusion area of 0.64 $cm^2$ was used to separate the donor and the receptor compartment. This kind of membrane allows the passage through for the drug and the cyclodextrin but not the polymer. Placed into the donor chamber was 3 mL of the system. In the receptor chamber 3 mL of phosphate buffer pH 6.8 (Newell et al., 1993) under continuous stirring were located. At preset times, samples were collected and the medium replaced by fresh buffer to keep *sink* conditions. βLAP released was determined spectrophotometrically at 257 nm. Release profiles were fitted to zero-order kinetics and drug release rates ($k$, $\mu g\,h^{-1}\,cm^{-2}$) were estimated from the slopes.

### 2.5. Transmission electron microscopy (TEM)

Selected systems were evaluated by TEM. Filtered systems (0.45 μm) were placed on TEM grids covered with Fomvar. After 30 s, the excess was removed and a drop of water was added. The excess was again removed after 30 s and a drop of 2 wt% phosphotungstic acid was added and left 30 s before removing. The systems were dried in a container with silica gel, and observed using a Philips CM-12 (FEI Company, The Netherlands).

### 2.6. Artificial Neural Networks modeling

A commercial implementation of a multi-layer perceptron neural network with several back-propagation learning algorithms INForm V4.1 (Intelligensys Ltd., UK) was used for modeling and optimizing the experimental data.

A total set of 34 experimental data consisting of systems of different composition were prepared (Table 1) and analyzed. RMβCD and PF127 concentrations (%, w/w) were considered as the inputs and solubilized βLAP and gel temperature were considered as outputs.

Dataset was divided randomly in three groups, 28 records for training the model, 3 records as test data to prevent overtraining (11, 18 and 26) and 3 records were preserved as the validation data (3, 14 and 20) to be used as unseen data and assess predictability.

Modeling was carried out selecting the parameters in Table 2 for the ANN training.

The accuracy of the Artificial Neural Network model was assessed using the ANOVA correlation coefficient ($R^2$) for each output.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2}$$

where $\bar{y}$ is the mean of the dependent variable, and $\hat{y}$ is the predicted value from the model.

The larger the value of the Train Set $R^2$, the better the model captured the variation in the training data. Values between 70 and 99.9% indicate reasonable model predictabilities (Colbourn and Rowe, 2009).

**Table 1**
Differential characteristics of the formulations studied and mean values of the parameters used to characterize them (standard deviations in parenthesis).

| Inputs | | | Outputs | | |
|---|---|---|---|---|---|
| Experiment | PF127 (%) | RMβCD (%) | Solubilized βLAP (μg/mL) | Enhancement Factor | $T_{gel}$ (°C) |
| | 0 | 0 | *38.57* | – | – |
| 1 | 0 | 0.5 | 270.04 (2.87) | 7 | Not gel at 37 °C |
| 2 | 0 | 0.8 | 403.51 (5.65) | 10.46 | Not gel at 37 °C |
| 3 | 0 | 1 | 527.56 (13.0) | 13.68 | Not gel at 37 °C |
| 4 | 0 | 1.5 | 869.45 (13.13) | 22.54 | Not gel at 37 °C |
| 5 | 0 | 2 | 1127.84 (58.41) | 29.24 | Not gel at 37 °C |
| 6 | 0 | 3 | 1757.49 (19.84) | 45.57 | Not gel at 37 °C |
| 7 | 0 | 5 | 3197.25 (139.48) | 82.89 | Not gel at 37 °C |
| 8 | 0 | 7 | 4708.81 (104.93) | 122.08 | Not gel at 37 °C |
| 9 | 10 | 0 | 48.37 (11.31) | 1.25 | Not gel at 37 °C |
| 10 | 10 | 3 | 971.84 (36.95) | 20.84 | Not gel at 37 °C |
| 11 | 10 | 5 | 1690.81 (93.49) | 36.26 | Not gel at 37 °C |
| 12 | 10 | 7 | 2300.50 (38.99) | 49.34 | Not gel at 37 °C |
| 13 | 15 | 0 | 75.60 (11.41) | 1.96 | 25.50 (0.06) |
| 14 | 15 | 3 | 698.22 (21.31) | 9.24 | 31.35 (0.03) |
| 15 | 15 | 5 | 1117.83 (118.01) | 14.79 | 33.75 (0.08) |
| 16 | 15 | 7 | 1869.25 (169.74) | 24.73 | 35.30 (0.10) |
| 17 | 20 | 0 | 108.37 (23.84) | 2.81 | 16.42 (0.05) |
| 18 | 20 | 3 | 676.03 (136.71) | 6.47 | 22.98 (0.04) |
| 19 | 20 | 5 | 1251.40 (48.47) | 11.98 | 23.18 (0.12) |
| 20 | 20 | 7 | 1894.51 (143.42) | 18.13 | 26.78 (0.06) |
| 21 | 30 | 0 | 184.85 (32.78) | 4.79 | Gel at 15 °C |
| 22 | 13 | 3 | – | – | 34.6 (0.15) |
| 23 | 13 | 7 | – | – | Not gel at 37 °C |
| 24 | 16.5 | 0 | – | – | 25.90 (0.02) |
| 25 | 16.5 | 3 | – | – | 30.25 (0.05) |
| 26 | 16.5 | 5 | – | – | 34.74 (0.02) |
| 27 | 16.5 | 7 | – | – | 36.15 (0.10) |
| 28 | 18.5 | 0 | – | – | 21.70 (0.06) |
| 29 | 18.5 | 3 | – | – | 25.93 (0.16) |
| 30 | 18.5 | 5 | – | – | 31.00 (0.07) |
| 32 | 18.5 | 7 | – | – | 35.11 (0.03) |
| 33 | 19 | 3 | – | – | 25.50 (0.04) |
| 34 | 19 | 7 | – | – | 28.50 (0.09) |

The italic value is the aqueous solubility of the drug.

## 3. Results and discussion

### 3.1. Neural network modellization

INForm® was successful on modeling and validating simultaneously the two parameters studied, βLAP solubilization and $T_{gel}$ that have been selected as essential criteria when the intratumoral formulation is designed. The ANOVA computed *f* value over 4 together with the training set $R^2$ and the test set $R^2$, far over 75%, indicates good predictability and performance of the model (Shao et al., 2006) (Table 3).

Good correlations ($r > 94$; slope close to 1) were found between experimental and predicted from the model results even for unseen data during training (validation data) (Fig. 1A and B) which corroborate the adequate predictability of the "black box" model for the two outputs simultaneously.

**Table 2**
Training parameters used for INForm modeling.

| | |
|---|---|
| Network structure | |
| No. hidden layers | 1 |
| No. nodes in hidden layer | 2 |
| Back propagation type | RPROP |
| Transfer function in | |
| Output transfer type | Linear |
| Hidden layer | Asymmetric sigmoid |
| Targets | |
| Targets epochs | 1000 |
| Target MS error | 0.0001 |
| Random seed | 10,000 |
| Test data | |
| Screen update set | 5 |
| Smart stop enabled | Toggled on |
| Minimum interactions | 20 |
| Test error weighting | 0.2 |
| Autoweight | Toggled on |

### 3.2. β-LAP solubilization

βLAP solubility in the systems and the corresponding Enhancement Factors (EF) are shown in Table 1. As it can be easily deduced, both PF127 and RMβCD increase the solubilization of the drug. Micellar structures of PF127 incorporated βLAP into their cores increasing the solubilized amount between 1.0 and 4.8 fold and RMβCD promoted solubilization amounts up to 122 fold. However, the βLAP solubilized amount when PF127 and RMβCD are used together in a formulation at the same concentration does not result in the addition of the corresponding βLAP solubilized amounts (additive effect) but a reduction in βLAP solubilization.

Fig. 2 shows the 3D plot of solubilized βLAP values predicted by the model as a function of percentages of PF127 and RMβCD in the formulation. The 3D plot points out interesting features about the interaction between products in ternary systems. The incorporation of βLAP into the surfactant micelles caused an increase in the solubility of the drug. However, at highest PF127 values (30%), the βLAP concentration still remained lower than 1 mg/mL (184.85 μg/mL), meaning that the use of micelle systems as a unique approach (Rao et al., 2006) (Fig. 2A) is not enough to improve βLAP solubilization at the desirable concentrations.

Higher βLAP solubilization through the formation of inclusion complexes was obtained when RMβCD was used, which is in agreement with previous authors (Cunha-Filho et al., 2007). The highest percentage of RMβCD (7%) allows the solubilization of nearly 5 mg/mL of βLAP. When PF127 is added to this solution to

**Table 3**
ANOVA results and correlation coefficients for the outputs studied.

| Output | Training data $R^2$ | Computed $f$ | d.f. | Test data $R^2$ | Validation data $R^2$ |
|---|---|---|---|---|---|
| Solubilized βLAP | 99.5625 | 175.2 | 9 and 16 | 83.8522 | 87.2981 |
| $T_{gel}$ | 96.0086 | 12.9 | 9 and 14 | 88.1563 | 95.5888 |

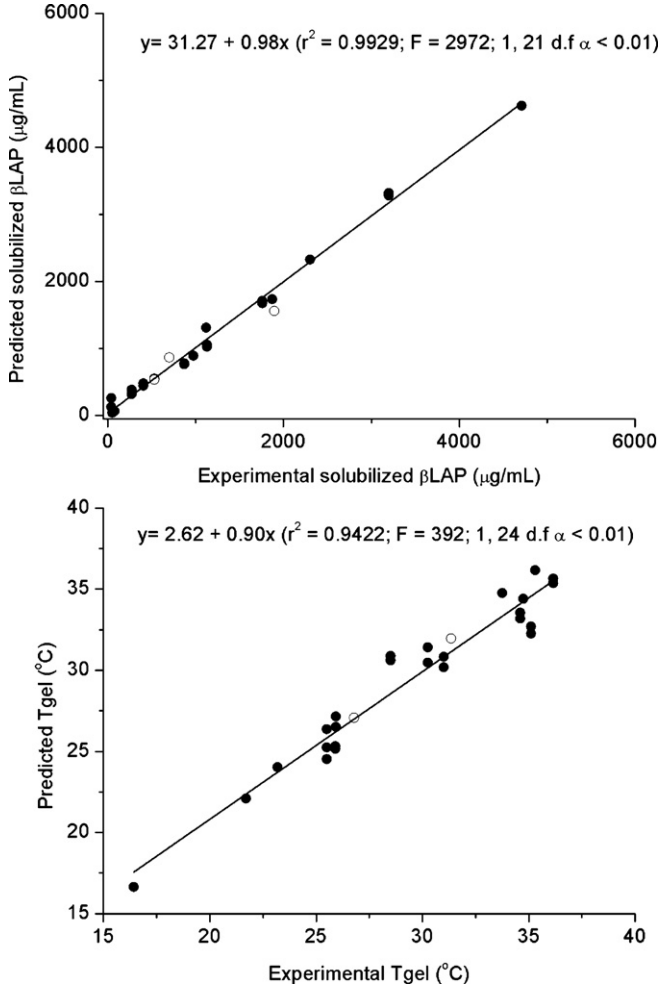


**Fig. 1.** Correlations between experimental and predicted values for the outputs studied (closed symbols correspond to training and test data, open symbols with unseen validation data).

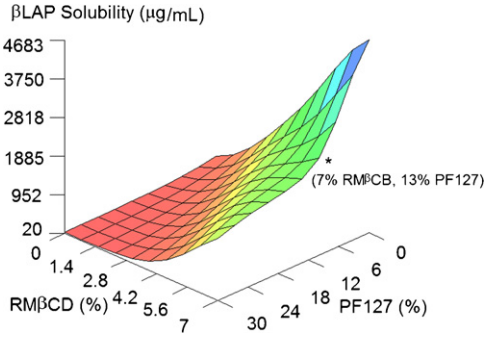


**Fig. 2.** 3D plot of solubility of βLAP predicted by the model.

provide thermo-sensitive properties, a dramatic reduction in the amount of solubilized drug takes place.

This phenomenon could be justified by the interaction between the pluronic and the RMβCD, which was pointed out by other authors (Valero and Dreiss, 2010), and the formation of soluble complexes named polypseudorotaxanes (Gaitano et al., 1997; Nogueiras-Nieto et al., 2009). The incorporation of PF127 to RMβCD/βLAP solutions gives the inclusion of PF127 chains into the cyclodextrin cavities, the competitive displacement of βLAP from the inside cavities and the decrease of the solubilization of the drug.

It is remarkable the presence of some inflexion points at the 3D βLAP solubility surface response (e.g. asterisk in Fig. 2). For solutions including 7%RMβCD, βLAP solubility enhancement decreases linearly with the addition of the polymer (EF = 122.45 – 5.98 [% PF127]; $r = 0.9978$ with 1 and 6 f.d. $\alpha < 0.01$) until approximately 13% PF127.

The model for βLAP solubility allowed interesting calculations to be made that corroborated the drug displacement by the polymer. The predicted βLAP solubility in a solution of RMβCD at 7% ($58.7 \times 10^{-5}$ moles per mL) is $1.93 \times 10^{-5}$ moles per mL. After the addition of 13% PF127 ($1.03 \times 10^{-5}$ moles per mL) the predicted βLAP solubility is $8.64 \times 10^{-6}$ moles per mL. The reduction in βLAP solubility, $1.05 \times 10^{-5}$ moles per mL, is really close to the molar concentration of the added polymer.

In order to investigate this phenomenon deeper; three systems including βLAP were prepared, as explained above, and analyzed by TEM. Their compositions were 7% RMβCD, 13% PF127 and their mixture 7% RMβCD–13% PF127 (Fig. 3A–C). The formation of organized aggregates of PF127 micelles (Fig. 3A) and RMβCD inclusion complexes in a supramolecular conformation (Fig. 3B) (Loftsson and Duchêne, 2007) incorporating βLAP can be observed. At the inflexion point (asterisk point at Fig. 2) the system is formed by cyclodextrin aggregates and just a few incipient micelles can be detected (Fig. 3C). The 7% RMβCD concentration leads to the complete disruption of PF127 micelles (Valero and Dreiss, 2010) the critical micellar concentration being higher than 13%. The

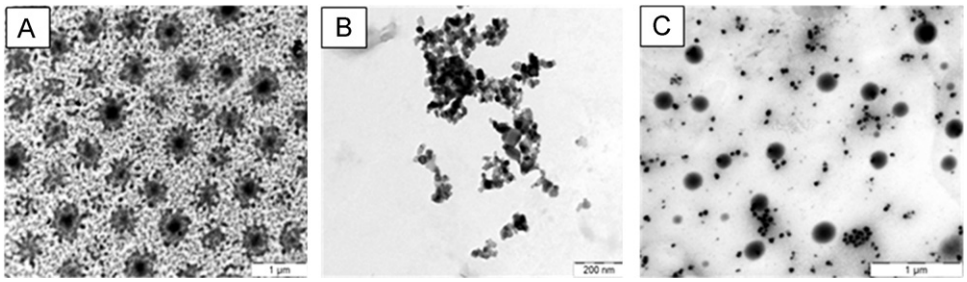


**Fig. 3.** TEM photomicrographs of systems with different compositions including βLAP (A) 13%PF127, (B) 7% RMβCD and (C) 13%PF127–7% RMβCD.

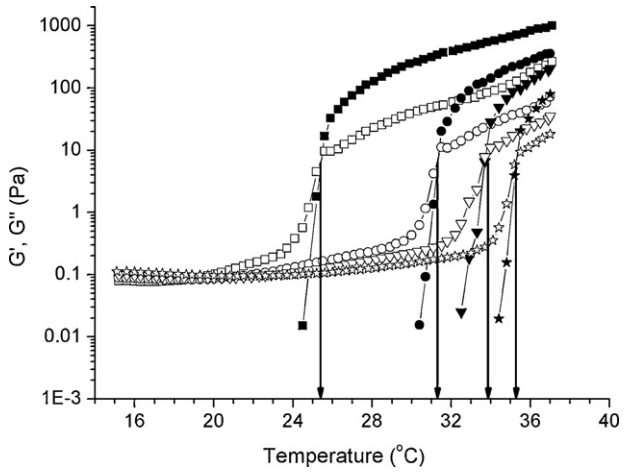


**Fig. 4.** Dynamic elastic ($G'$) and viscous ($G''$) moduli as function of temperature for formulations including 15% FP127: (■, □) 0% RMβCD, (●, ○) 3% RMβCD, (▼, ▽) 5% RMβCD and (★, ☆) 7% RMβCD (closed symbols: $G'$; open symbols: $G''$).

formation of new micelles over 13% of PF127 helps to explain the change in the slope.

### 3.3. Rheological characterization

A well designed intratumoral system should be a viscous solution at room temperature and a gel at physiological temperature (37 °C) forming a depot from where the drug is released at a controlled rate.

Temperature has an important effect on pluronic solutions. Micelle orientation change when the temperature increases resulting in an enhancement in system consistency and gelation (Chaibundit et al., 2008). It is well known that additives (co solvents, surfactants) and drugs can modify rheological behavior of pluronic systems (Bonacucina et al., 2007; Cohn et al., 2005; Kadam et al., 2011; Valero and Dreiss, 2010). Our results agreed with previous authors. The rheological profile at 15 °C for all the PF127 systems, except for 30% PF127, were typical of polymeric solutions at a concentration below the critical gelling concentration; viscosity values in the range 0.01–0.05 Pa s/rad, $G'' \gg G'$ and a strong frequency dependence in the moduli. At this temperature PF127 systems below 20% concentration remain fluid injectable solutions.

At physiological temperature (37 °C), all the systems over 13% PF127 showed rheological profiles characterized by a pronounced plateau with $G'$ over $G''$ ($G'$ and $G''$in the range $10^2$–$10^4$ Pa and 10–$10^2$ Pa respectively) and meaning that they fulfill Almdal et al. (1993) requirements to be considered as hard gels.

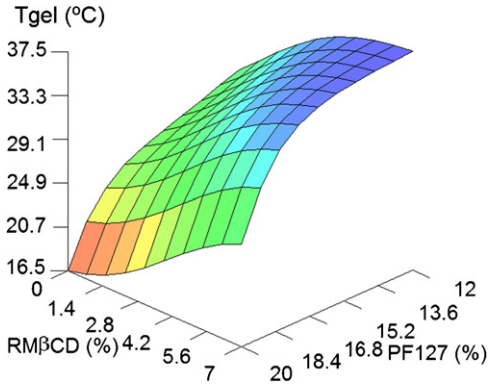


**Fig. 5.** 3D plot of gel temperature of the systems predicted by the model.

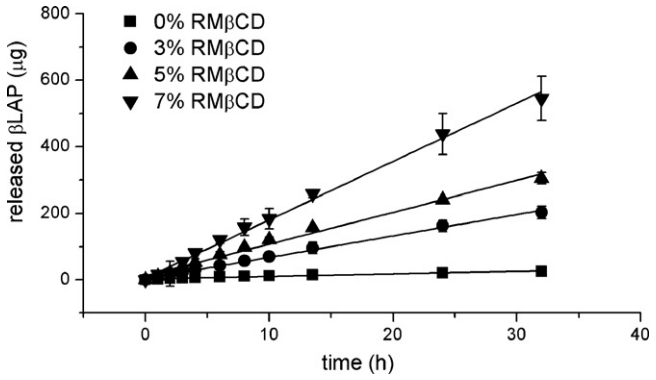


**Fig. 6.** βLAP release profile from 15% PF127 systems including increasing percentages of RMβCD.

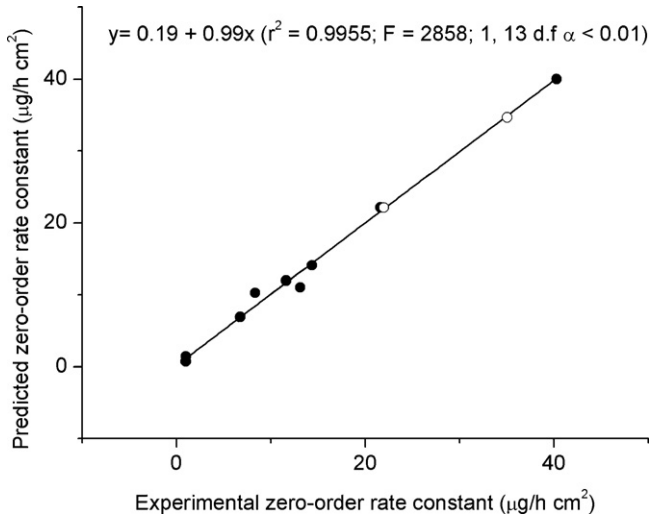


**Fig. 7.** Correlations between experimental and predicted values for the output zero-order rate constant (closed symbols correspond to training and open symbols with test data).

RMβCD has a strong influence on PF127 system rheological characteristics. As an example, we have presented the ramps of temperature of several systems developed with 15% PF127 and increasing concentrations of RMβCD in Fig. 4. As it can be seen, the cross point between $G'$ and $G''$ indicative of its $T_{gel}$ (indicated with arrows) has the highest value as the system incorporates RMβCD. This effect, previously described for PF127 and HPβCD or methyl βCD combinations (Chaibundit et al., 2008; Valero and Dreiss, 2010) supported the hypothesis of polypseudorotaxanes formation. The RMβCD increases critical micellar concentration of

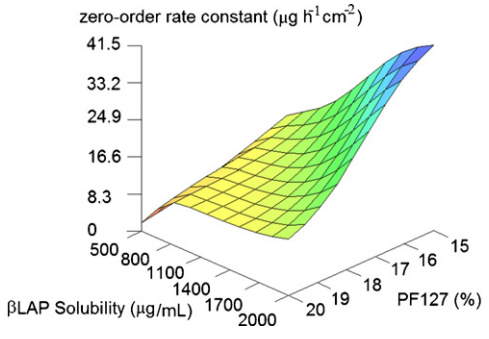


**Fig. 8.** 3D plot of release zero-order βLAP release rate constant predicted by the model.

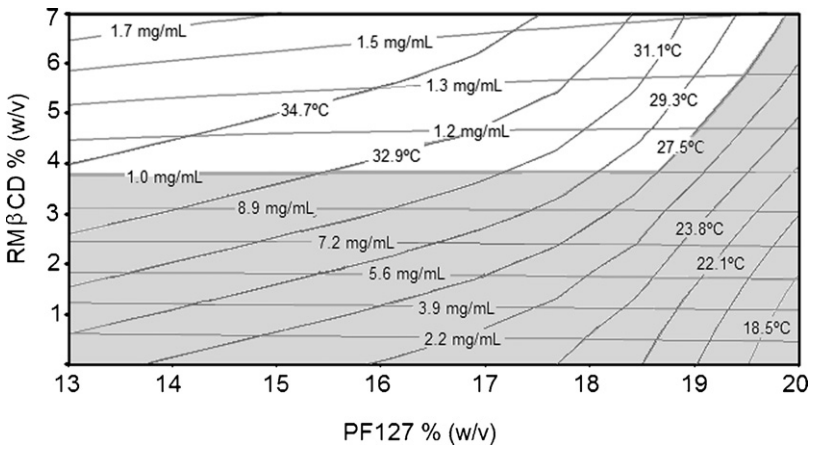


**Fig. 9.** Contour plot of the influence of percentage of RMβCD and PF127 on βLAP solubilization (mg/mL) and gelation temperature (°C). White area indicates formulations design space.

PF127 as the hydrophobic chains included in the CD cavity cannot be involved in the micellation process.

3-D plot of the predicted $T_{gel}$ temperatures by ANN (Fig. 5) let us conclude that the addition of RMβCD to the systems containing PF127 caused a non linear increase in the gel temperature related to the formation of polypseudorotaxanes aggregates (Nogueiras-Nieto et al., 2009). Additionally, the $T_{gel}$ drops dramatically when the percentage of PF127 in the formulation is higher that 17%.

### 3.4. In vitro release assays

To be considered as implants the systems must release the βLAP in a controlled manner for a long period of time. Diffusion is considered to be the main release mechanism for intratumoral implants (Amiji et al., 2002; Weinberg et al., 2007).

βLAP releases from different systems having adequate syringeability and gel temperature were studied using horizontal diffusion cells. As an example, we present the βLAP release profiles of several systems developed with 15% PF127 and increasing concentrations of RMβCD in Fig. 6. During the first 30 h all fit zero order kinetics ($r^2 > 0.91$ with 1 and 9 d.f. and $F > 45$; $\alpha < 0.01$). Zero-order rate constants were calculated from the corresponding slopes.

In order to make predictions zero-order constant rates calculated from the corresponding slopes were modeled by INForm using the same training parameters (Table 2) as the previous model but introducing the solubilized βLAP in the system and the percentage of PF127 as inputs. Good ANOVA correlation coefficients were also found for training data ($R^2 = 99.2908$) and test data ($R^2 = 99.8194$) and also good correlation between predicted experimental data were obtained (Fig. 7).

InForm model also shows a complex non linear relationship between inputs for the release rate of the drug (Fig. 8) dramatically decreasing when highest amounts of polymer are added to the systems.

### 3.5. Design space of pluronic F127®/RMβCD/β-LAP formulations

The combination of the surface responses from the ANN model for the parameters studied (Fig. 9) helps to establish the design space of this type of formulation.

Two main considerations may be taking into account for intratumoral formulations. The formulation should include a dose of drug which can be controlled released for a prolonged period of time and also be a gel at body temperature.

βLAP has been applied against many different cancer cells in culture, finding that lethal concentration varies in the range of 1–30 μM (Li et al., 2003) but there is no accurately available information regarding the dose of βLAP or the release rate for effective intratumorally depot systems of this drug. Dong et al. (2009) achieved, during in vivo experiments in mice, antitumor efficacy on some types of prostate cancer cell lines with intratumoral formulations of βLAP. Those implants included a dose of βLAP of 1 mg released through a period of 20 days.

For the ternary systems proposed in this paper, the formulations should contain over 3.5% of RMβCD to achieve a dose higher than 1 mg/mL (Fig. 9). Simultaneously, the percentage of PF127 should be over 18.5% for the systems to have a temperature of gelation over 27 °C. This property should make easy their handling, being syringeable at room temperature and gelling quickly after injection. Additionally, those systems will control the drug release for several days. Experiments are being carried out in order to corroborate the in vivo therapeutic effect of βLAP from those systems on MCF-7 human ectopic breast tumors (Table 4).

**Table 4**
Example of optimization conditions.

| Property | Weight | Function | Min | Mid1 | Mid2 |
|---|---|---|---|---|---|
| Solubilized βLAP | 10 | More than | 1000 | 1501 | 1502 |
| $T_{gel}$ | 9 | Between | 27 | 31 | 32 |

## 4. Conclusions

ANN software was successful in modeling simultaneously two important parameters of injectable thermogelling formulations of βLAP; the solubilization of the drug into the system and its gel temperature. Its predictability was assessed by the good fit of the validation data set.

The ANN model allowed an understanding of the interactions between components in the ternary system (βLAP-F127-RMβCD) and the detecting and quantifying of fundamental phenomena, such as the formation of polypseudorotaxanes and/or the modification of critical micellar concentration.

Our results show that the artificial intelligence methods used in this paper address and characterize the design space of the formulation without the restrictions imposed by experimental designs, thus giving the formulator new opportunities to faster develop and test better formulations.

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## References

Al-Abd, A.M., Hong, K.-Y., Song, S.-C., Kuh, H.-J., 2010. Pharmacokinetics of doxorubicin after intratumoral injection using a thermosensitive hydrogel in tumor-bearing mice. J. Control. Release 142, 101–107.

Almdal, K., Dyre, J., Hvidt, S., Kramer, O., 1993. Towards a phenomenological definition of the term 'gel'. Polym. Gels Networks 1, 5–17.

Amiji, M.M., Lai, P.-K., Shenoy, D.B., Rao, M., 2002. Intratumoral administration of paclitaxel in an in situ gelling Poloxamer 407 formulation. Pharm. Dev. Technol. 7, 195–202.

Arulsudar, N., Subramanian, N., Murthy, R.S.R., 2005. Comparison of artificial neural network and multiple linear regression in the optimization of formulation parameters of leuprolide acetate loaded liposomes. J. Pharm. Pharm. Sci. 8, 243–258.

Bey, E.A., Bentle, M.S., Reinicke, K.E., Dong, Y., Yang, C.-R., Girard, L., Minna, J.D., Bornmann, W.G., Gao, J., Boothman, D.A., 2007. An NQO1- and PARP-1-mediated cell death pathway induced in non-small-cell lung cancer cells by β-lapachone. Proc. Natl. Acad. Sci. U. S. A. 104, 11832–11837.

Blanco, E., Bey, E.A., Dong, Y., Weinberg, B.D., Sutton, D.M., Boothman, D.A., Gao, J., 2007. β-Lapachone-containing PEG-PLA polymer micelles as novel nanotherapeutics against NQO1-overexpressing tumor cells. J. Control. Release 122, 365–374.

Bonacucina, G., Spina, M., Misici-Falzi, M., Cespi, M., Pucciarelli, S., Angeletti, M., Palmieri, G.F., 2007. Effect of hydroxypropyl-cyclodextrin on the self-assembling and thermogelation properties of Poloxamer 407. Eur. J. Pharm. Sci. 3 (2), 115–122.

Chaibundit, C., Ricardo, N.M., Costa, F.M.L.L., Wong, M.G., Hermida-Merino, D., Rodriguez-Perez, J., Hamley, I.W., Yeates, S.G., Booth, C., 2008. Effect of ethanol on the micellization and gelation of Pluronic P123. Langmuir 24, 12260–12266.

Cohn, D., Sosnik, A., Garty, S., 2005. Smart hydrogels for in situ generated implants. Biomacromolecules 6, 1168–1175.

Colbourn, E.A., Rowe, R.C., 2009. Novel approaches to neural and evolutionary computing in pharmaceutical formulation: challenges and new possibilities. Future Med. Chem. 1 (4), 719–726.

Cunha-Filho, M.S., Dacunha-Marinho, B., Torres-Labandeira, J.J., Martínez-Pacheco, R., Landín, M., 2007. Characterization of β-lapachone and methylated β-cyclodextrin solid-state systems. AAPS PharmSciTech 8 (3), E1–E10.

Dong, Y., Chin, S.F., Blanco, E., Bey, E.A., Kabbani, W., Xie, X.J., Bornmann, W.G., Boothman, D.A., Gao, J., 2009. Intratumoral delivery of beta-lapachone via polymer implants for prostate cancer therapy. Clin. Cancer Res. 15, 131–139.

Donghui, Z., Xiaolin, L., Huae, X., Xiaowei, L., Yong, H., Weixin, F., 2009. Study on docetaxel-loaded nanoparticles with high antitumor efficacy against malignant melanoma. Arch. Biochem. Biophys. 41, 578–587.

Dreiss, C.A., Nwabunwanne, E., Liu, R., Brooks, N.J., 2009. Assembling and de-assembling micelles: competitive interactions of cyclodextrins and drugs with Pluronics. Soft Matter 5, 1888–1896.

Ferreira, D.C.M., Tapsoba, I., Arbault, S., Bouret, Y., Moreira, M.S.A., Pinto, A.V., Goulart, M.O.F., Amatore, C., 2009. Ex vivo activities of β-lapachone and α-lapachone on macrophages: a quantitative pharmacological analysis based on amperometric monitoring of oxidative bursts by single cells. ChemBioChem 10, 528–538.

French, J.T., Goins, B., Saenz, M., Li, S., Garcia-Rojas, X., Phillips, W.T., Otto, R.A., Bao, A., 2010. Interventional therapy of head and neck cancer with lipid nanoparticle-carried rhenium 186 radionuclide. J. Vasc. Interv. Radiol. 21 (8), 1271–1279.

Gaitano, G.G., Brown, W., Tardajos, G., 1997. Inclusion complexes between cyclodextrins and triblock copolymers in aqueous solution: a dynamic and static light-scattering study. J. Phys. Chem. B 101, 710–719.

Gupta, P.K., 1990. Drug targeting in cancer chemotherapy: a clinical perspective. J. Pharm. Sci. 79, 949–962.

Hwang, T.L., Lee, W.R., Hua, S.C., Fang, J.L., 2007. Cisplatin encapsulated in phosphatidylethanolamine liposomes enhances the in vitro cytotoxicity and in vivo intratumor drug accumulation against melanomas. J. Dermatol. Sci. 46, 11–20.

Jeong, B., Bae, Y.H., Lee, D.S., Kim, S.W., 1997. Biodegradable block copolymers as injectable drug-delivery systems. Nature 28, 860–862.

Jeong, S.Y., Park, S.-J., Yoon, S.M., Jung, J., Woo, H.N., Yi, S.L., Song, S.Y., Park, H.J., Kim, C., Lee, J.S., Lee, J.S., Choi, E.K., 2009. Systemic delivery and preclinical evaluation of Au nanoparticle containing β-lapachone for radiosensitization. J. Control. Release 139, 239–245.

Johnston, T.P., Miller, S.C., 1985. Toxicological evaluation of poloxamers for intramuscular use. J. Parent. Sci. Technol. 39, 83–88.

Kabanov, A.V., Batrakova, E.V., Alakhov, V.Y., 2002a. Pluronic® block copolymers for overcoming drug resistance in cancer. Adv. Drug Deliv. Rev. 54, 759–779.

Kabanov, A.V., Batrakova, E.V., Alakhov, V.Y., 2002b. Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery. J. Control. Release 82, 189–212.

Kadam, Y., Yerramilli, U., Bahadur, A., Bahadur, P., 2011. Micelles from PEO–PPO–PEO block copolymers as nanocontainers for solubilization of a poorly water soluble drug hydrochlorothiazide. Colloid Surf. B: Biointerfaces 83, 49–57.

Karasulu, H.Y., Karabulut, B., Kantarci, C., Özgüney, I., Sezgin, C., Sanli, U.A., Göker, E., 2004. Preparation of arsenic trioxide-loaded microemulsion and its enhanced cytotoxicity on MCF-7 breast carcinoma cell line. Drug Deliv. 11, 345–350.

Landin, M., Rowe, R.C., York, P., 2009. Advantages of neurofuzzy logic against conventional experimental design and statistical analysis in studying and developing direct compression formulations. Eur. J. Pharm. Sci. 38, 325–331.

Li, Y., Sun, X., LaMont, J.T., Pardee, A.B., Li, C.J., 2003. Selective killing of cancer cells by β-lapachone: direct checkpoint activation as a strategy against cancer. Proc. Natl. Acad. Sci. U. S. A. 100, 2674–2678.

Loftsson, T., Duchêne, D., 2007. Cyclodextrins and their pharmaceutical applications. Int. J. Pharm. 329, 1–11.

Nasongkla, N., Wiedmann, A.F., Bruening, A., Beman, M., Ray, D., Bornmann, W.G., 2003. Enhancement of solubility and bioavailability of beta-lapachone using cyclodextrin inclusion complexes. Pharm. Res. 20, 1626–1633.

Newell, K., Franchi, A., Pouysségur, J., Tannock, I., 1993. Studies with glycolysis-deficient cells suggest that production of lactic acid is not the only cause of tumor acidity. Proc. Natl. Acad. Sci. U. S. A. 90, 1127–1131.

Nogueiras-Nieto, L., Alvarez-Lorenzo, C., Sanchez-Macho, I., Concheiro, A., Otero-Espinar, F., 2009. Hydrosoluble cyclodextrin/poloxamer polypseudorotaxanes at the air/water interface, in bulk solution, and in gel state. J. Phys. Chem. B 113, 2773–2782.

Ough, M., Lewis, A., Bey, E.A., Gao, J., Ritchie, J.M., Bornmann, W., Boothman, D.A., Oberley, L.W., Cullen, J.J., 2005. Efficacy of beta-lapachone in pancreatic cancer treatment: exploiting the novel, therapeutic target NQO1. Cancer Biol. Ther. 4 (1), 95–102.

Park, H.J., Ahn, K., Ahn, S., Choi, E., Lee, S.W., Williams, B., Kim, E.J., Griffin, R., Bey, E.A., Bornmann, W.G., Gao, J., Park, H.J., Boothman, D.A., Song, C.W., 2005. Susceptibility of cancer cells to β-lapachone is enhanced by ionizing radiation. Int. J. Radiat. Oncol. Biol. Phys. 61, 212–219.

Park, I.K., Kim, K.K., Cho, S.H., Bae, W.K., Jere, D., Cho, C.S., Chung, I.J., 2010. Intratumoral administration of anti-KITENIN shRNA-loaded PEI-alt-PEG nanoparticles suppressed colon carcinoma established subcutaneously in mice. J. Nanosci. Nanotechnol. 10 (5), 3280–3283.

Ranganath, H.S., Fu, Y., Arifin, D.Y., Kee, I., Zheng, L., Lee, H.-S., Chow, P.K.-H., Wang, C.-H., 2010. The use of submicron/nanoscale PLGA implants to deliver paclitaxel with enhanced pharmacokinetics and therapeutic efficacy in intracranial glioblastoma in mice. Biomaterials 31, 5199–5207.

Rao, V., Nerurkar, M., Pinnamaneni, S., Rinaldi, F., Raghavan, K., 2006. Co-solubilization of poorly soluble drugs by micellization and complexation. Int. J. Pharm. 319, 98–106.

Shao, Q., Rowe, R.C., York, P., 2006. Comparison of neurofuzzy logic and neural networks in modelling experimental data of an immediate release tablet formulation. Eur. J. Pharm. Sci. 28, 394–404.

Simões, S.M.N., Veiga, F., Torres-Labandeira, J.J., Ribeiro, A.C.F., Sandez-Macho, M.I., Concheiro, A., Alvarez-Lorenzo, C., 2012. Syringeable Pluronic-a-cyclodextrin supramolecular gels for sustained delivery of vancomycin. Eur. J. Pharm. Biopharm. 80, 103–112.

Takahashi, T., Ueda, S., Kono, K., Majima, S., 1976. Attempt at local administration of anticancer agents in the form of fat emulsion. Cancer 38, 1507–1514.

Takayama, K., Fujikawa, M., Nagai, T., 1999. Artificial neural network as a novel method to optimize pharmaceutical formulations. Pharm. Res. 16, 1–6.

Valero, M., Dreiss, C.A., 2010. Growth, shrinking, and breaking of pluronic micelles in the presence of drugs and/or β-cyclodextrin, a study by small-angle neutron scattering and fluorescence spectroscopy. Langmuir 26, 10561–10571.

Van Tomme, S.R., Storm, G., Hennink, W.E., 2008. In situ gelling hydrogels for pharmaceutical and biomedical applications. Int. J. Pharm. 355, 1–18.

Wang, F., Blanco, E., Ai, H., Boothman, D.A., Gao, J., 2006. Modulating β-lapachona release from polymer millrods through cyclodextrin complexation. J. Pharm. Sci. 95 (10), 2309–2319.

Weinberg, B.E., Blanco, E., Gao, J., 2007. Polymer implants for intratumoral drug delivery and cancer therapy. J. Pharm. Sci. 97, 1681–1702.

Yang, L., Wang, B., Qiau, W., Liu, P., 2009. A novel combination chemotherapy integrating with intratumoral chemotherapy. Med. Hypotheses 73, 334–335.