# Lyophilization of Imunoparvum® as an alternative to reduce its side-effects

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Abstract: *In Brazil, Imunoparvum® is used as an immunomodulator. It is commercialized in 2 ml ampoules containing 2 mg of Propionebacterium acnes (formerly known as Corynebacterium parvum)/ml, in a 0.5 % phenol and 0.85% sodium chloride solution. High therapeutic power associated to minimum side effects is a great challenge to the pharmaceutical industry. Research results have shown that Imunoparvum® induces side effects in humans and animals, probably because of its phenol content (SOUSA, 1993; RODRIGUES, 2001). The objective of this study was to determine the phenol content of lyophilized and non-lyophilized Imunoparvum® and to compare their side effects in mice. It was demonstrated that the lyophilization process reduces the phenol content and the side effects of Imunoparvum®, when compared to the commercialized Propionebacterium acnes suspension.*

Key words: *Imunoparvum®, hypersensitivity, cancer, adjuvant, phenol*

Resumo: *No Brasil, o Imunoparvum® é usado como imunomodulador. É envasado em ampolas contendo 2 mg/ml de Propioniobacterium acnes (antigo Corynebacterium parvum) em*

*uma suspensão de cloreto de sódio contendo 0,5 % de fenol. Um aumento da sua eficiência terapêutica associada à diminuição dos seus efeitos colaterais é um grande desafio à indústria farmacêutica. Pesquisas têm mostrado que o Imunoparvum® induz efeitos colaterais em humanos e em animais, provavelmente em virtude da presença do fenol como parte integrante da formulação comercial (SOUSA, 1993; RODRIGUES, 2001). O objetivo deste estudo foi analisar as concentrações de fenol no Imunoparvum® em suspensão após submetê-lo a processo de liofilização, comparando os seus efeitos colaterais em camundongos. Os resultados encontrados no presente modelo revelaram que a liofilização reduziu tanto os teores de fenol quanto os efeitos colaterais, quando comparados à forma em suspensão.*

Palavras-chave: *Imunoparvum®, hipersensitividade, câncer, fenol, adjuvante*

# 1 Introduction

Evidence has shown that *P. acnes* is capable of enhancing the immune response in human and animals, modulating the resistance to infections and increasing the efficiency when associated at conventional therapy of several diseases (CASTRO, 1974, TEIXEIRA, 1996, SOUSA, 1993, AEBISCHER, 2000). There are, however, several reports of human patients treated with *P. acnes* that developed adverse effects, such as pain after injection, skin irritation, fever and systemic shock. These observations are in agreement with the side effects observed in mice immediately after inoculation with suspension of *P. acnes* (SOUSA, 1993). Evidence demonstrated that these side effects have origin in the production of the vaccine, which maintain a high concentration of phenol in the formulation (SOUSA, 1993, RODRIGUES, 2001). The objective of this work was to use the lyophilization process as means to neutralize the phenol level in the suspension of Imunoparvum® after industrialization in order to reduce its side effects.

# 2 Methods

## 2.1 Determination of phenol content and cell concentration of P. acnes in Imunoparvum®

Non-lyophilized Imunoparvum® (NLI) suspension sample from 2 ml ampoule was filtered in sintered glass micro filter (5 ml capacity and porosity number 3). The resulting filtered solution was completed to one liter by the addition of distilled and deionized water (dd-water) and the solid phase was discarded. Lyophilized Imunoparvum® (LI) 4 mg samples were suspended in 5 ml of dd-water followed by a 30-minute period in a magnetic stirring apparatus. Then, the solution was filtered in sintered glass micro filter (5 ml capacity and porosity number 3) and the filtered solution was diluted to 50 ml of dd-water. Phenic acid concentration was determined by UV/VIS spectroscopy, measured at 210 nm wavelength, based on a 190 to 500 nm range scanning of a phenol standard solution, using dd-water as the reference.

Quality control tests were based in 100 ampoules of NLI and 100 ampoules of LI. The studied characteristics were the final volume, pH, aspect of the ampoule content, mean weight, phenol research by ferric acid method, syringe flow and *P. acnes* cell concentration, performed on both NLI and LI samples, in order to evaluate the effect of the lyophilization process.

### 2.2 *In vivo* trials

*In vivo* side-effects trials were carried out in nine groups of five Swiss mice. Each animal received a single 0.2 ml intra peritoneal dose. Group 1 mice received each one 0.2 mg of NLI solution. Groups 2, 3 and 4 mice received, respectively, 0.2 mg, 0,4 mg, and 1 mg of LI, dissolved in a 0.85% sodium chloride solution. Each mice from group 5 received 0.2 ml of a 0.85% sodium chloride solution with 0.5 % phenol concentration. Animals from groups 6 to 9 received 0.2 ml diluted (1/10, 1/100, 1/1000 and 1/10000, respectively) doses of the solution used in group 5 mice. Mice were observed during 72 hours after drug administration.

## 3 Results and discussion

A phenol solution, 0.0268 g/l, was submitted to a 190 to 500 nm UV/VIS spectroscopy scanning (Figure 1, curve 3). Maximum absorbance peaks were observed at 210 nm (characteristic of aromatic rings) and 270 nm (characteristic of −OH phenolic groups).

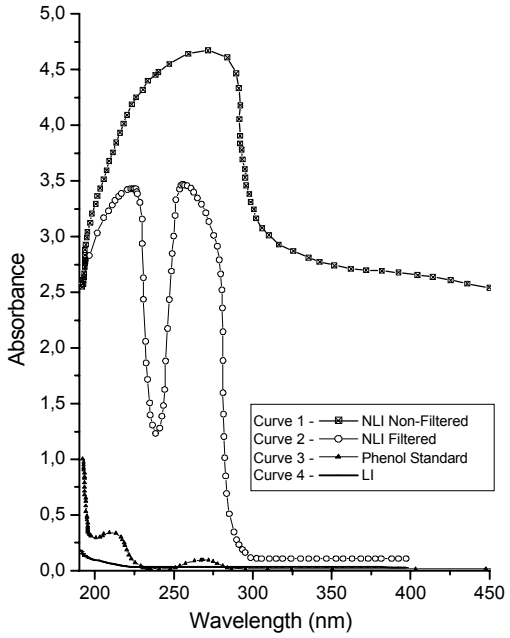


Figure 1. UV/VIS curves of investigated samples of NLI Non-Filtered (curve 1), NLI Filtered (curve 2), Phenol Standard (curve 3) and LI (curve 4).

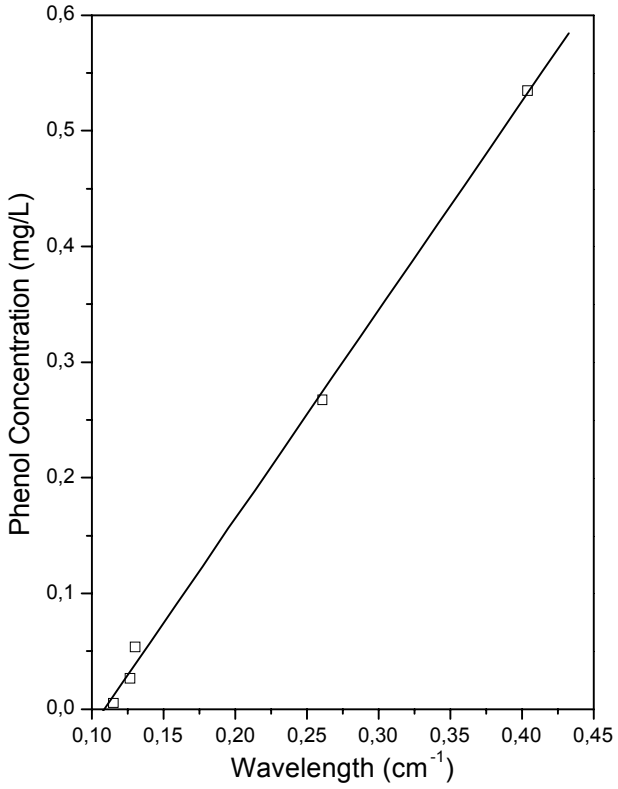


Figure 2. Standard curve used for determination of phenol concentration at 210 nm.

The standard curve was determined using five phenol solutions (535.00; 267.50; 53.50; 26.75 and 5.35 mg/ml). Absorbances were determined at 210 nm (Figure 2). Estimated linear regression equation was $C = -1.964 \times 10^{-4} + 18.000 \times 10^{-4}$ ABS ($r^2 = 0.998$). $C$ is the phenol concentration in g/l, and ABS is the absorbance at 210 nm.

Figure 1 shows the UV/VIS curves of the investigated samples. Results indicate that lyophilization decreases the phenol content of Imunoparvum® (Figure 1, curve 4). The solid phase of non-filtered NLI showed a hiding effect, as it does not permit to detect the 210 and 270 nm absorbance peaks observed in the filtered NLI, necessary to the determination of the phenol concentration (Figure 1, curve 1). After filtration and elimination of the solid phase, it was observed that the filtered NLI curve presented two peaks, corresponding to the phenolic absorbance (Figure 1, curve 2). This result indicated the high phenol content in NLI. LI curve (Figure 1, curve 4) confirms lyophilization efficiency in reducing the phenol content of Imunoparvum®. It was observed that the 0.51% phenol content in NLI was reduced to 0.004% in LI. The lyophilization process did not affect dry weight, pH, sterility and syringe flow of the re-hydrated Imunoparvum® (See Table 1).

| Analysis | NLI(ml) | LI | NaCl (0,85%) |
|---|---|---|---|
| Declared volume | 2.00 | | |
| Actual volume | 2.05 | | |
| pH | | | |
| Flasks with colorless content | | 6.26 | |
| Flasks with cloudy content | | 6.27 | |
| Supplied salt bed | | | 7.03 |
| Mean weight (mg/ml) | | 1.842 | |
| Variation of mean weight in 40% of the samples (g) | 0.0077 − 0.0229 | | |
| Variation of mean weight in 60% of the samples (g) | 0.02004 − 0.0229 | | |
| Phenol content by ferric chloride method | | | |
| NLI *in natura* Flasks | Positive | | |
| Flasks with colorless content | | Negative | |
| Flasks content slightly cloudy | | Fainly positive | |
| Syringe flow | Satisfies | Satisfies | |

Table 1. Physical chemical analysis of Imunoparvum®.

## 4 Clinical aspects

The 0.2 mg NLI dose caused slight skin irritation in the injection spot, accompanied by pain manifested by abdominal contortions, tachypnea, involuntary urination, and defecation in some of them. The symptoms disappeared within 30 to 40 minutes. Isolation or grouping behavior, depression, sweating and hair bristling were observed during 5 to 8 hours after drug administration. These same conditions were observed in all mice inoculated with diluted phenol only. Clinical aspects showed in this experiment are in accordance with Sousa (1993). Animals inoculated with LI did not present any side effects. Neither NLI nor LI killed the studied mice.

## 5 Conclusion

Lyophilization reduced the phenol content of Imunoparvum®. The present study also showed that phenol in high concentration seems to be responsible for the observed Imunoparvum® side effects in mice.

## Acknowledgements

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