# STUDY OF RESVERATROL STABILITY IMMOBILIZATION ON POLY VINYL PYRROLIDONE HYDROGEL DRESSING FOR DERMATOLOGICAL USE

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**Abstract**

The polyphenol trans-resveratrol is a natural phytoalexin, which is found in red wine and in a wide variety of plant species. Resveratrol has been shown wide biological activities, such as modulation of lipid metabolism, anti-inflammatory and antioxidant activities. This active compound immobilized in PVP (poly-vinyl pyrrolidone) hydrogel could be very interesting to a topical administration, as hydrogel dressing for dermatological use. However, PVP hydrogel obtained by gamma radiation crosslinking process can cause undesirable hydrolysis reactions in the active compound.
The aim of this work was to verify the resveratrol stability after irradiation at 0.5 and 1 kGy in the presence of ethanol, methanol or *tert*-Butyl alcohol and the integrity of them were compared with nature one by HPLC technique.
The PVP hydrogel matrix were characterized by gel fraction, swelling and *in vitro* test of biocompatibility.
The results of gel fraction of 90%, swelling degree of approximately 1600% and no cytotoxic effect in the cytotoxicity assay indicated that the PVP hydrogel formulation was appropriate for immobilization of resveratrol to produce a dressing for dermatological use.

**Keywords:** Trans-resveratrol, PVP hydrogel, HPLC.

## INTRODUCTION

The polyphenol trans-resveratrol (*trans*-3,4',5-trihydroxystilbene) is naturally occurring phytoalexin synthesized by a wide range of plant species, including *Vitis vinifera* and a variety of medicinal plants, in response to infection or injury. This compound has been shown to possess exceptionally activities and to have important biological, pharmacological and medicinal properties, such as inhibition of lipid peroxidation and platelet aggregation, vasorelaxing activity, anti-inflamatory and antioxidant activity (Iacopini *et. al*, 2008; Fremont, 2000).

Highly reactive molecules are present in biological systems and may oxidize nucleic acids, proteins, lipids, which may initiate degenerative process such as dermal disorders and aging. Trans-resveratrol can reduce this risk, because it acts as free radical scavenging, promoting the skin aging prevention (Iacopini *et. al*, 2008).

Polymeric hydrogels have a three-dimensional network structure and can swell considerably in aqueous medium without dissolution. Hydrogel crosslinking can be obtained by chemical initiator or by ionizing radiation. The radiation crosslinking process presents advantages in relation to chemical reactions but can cause undesirable hydrolysis reactions modifying the immobilized compound activity (Peppas *et. al*, 1996).

Polyvinyl pyrrolidone (PVP) has been used successfully as a basic material for the manufacturing of hydrogels. These types of hydrogel can be used as a drug delivery system due to biocompatibility, swelling capacity, easy dispersion of different active compounds and control of solute transport (Ajji *et. al*, 2005; Geever *et. al*, 2008).

The aim of the present study was to verify the irradiated resveratrol stability aiming this active compound immobilization in a PVP hydrogel matrix to obtain a resveratrol dressing for dermatological use.

The PVP matrix was characterized by swelling, gel fraction and *in vitro* test of biocompatibility.

## MATERIALS AND METHODS

Reagents PVP K-90 (Kollidon®) from BASF, PEG300 from Oxiteno, Agar from Oxoid, trans-resveratrol from Attivos Magistrais, Ethanol and Methanol from Vetec and *tert*-Butyl alcohol from Merck.

**Preparation of polymeric matrix**

The polymeric matrix was composed by 6% PVP K90, 1.5% PEG and 0.5% agar. The membranes were obtained in a circular mould, sealed and sent for irradiation in $^{60}$Co gamma ray source gamma with 10 kGy.h$^{-1}$ dose rate at 20 kGy dose.

**Swelling assay**

The swelling assays were accomplished in triplicate using phosphate buffered saline solution (PBS) pH 5. The samples were immersed in 30 mL PBS during 24 h and weighed every hour during the 6 first hours and after 24 h. Degrees of swelling were calculated by the Eq (1):

$$\text{swelling}\,(\%) = \frac{w_s - w_i}{w_i} \text{ x } 100 \qquad (1)$$

where $w_i$ is the initial dried sample weight and $w_s$ is the weight of sample after swelling

**Gel fraction**

The gel fraction assay was performed in triplicate using dried samples. The extraction of the soluble fraction was accomplished with distilled water in Sohxlet extractor during 40 h. After this period, the samples were dried until they reached constant weight and their mass was determined. Gel fraction was calculated by the Eq. (2):

$$\text{Gel fraction}\,(\%) = \frac{w_e}{w_i} \text{ x } 100 \qquad (2)$$

where $w_i$ and $w_e$ are the dried weight of the sample before and after extraction, respectively.

**Cytotoxicity assay**

The cytotoxicity test was carried out using NCTC clone 929 cell line from American Type Culture Collection (ATCC), according to the International Standardization Organization (ISO 10993-5, 1992) and the previous described methodology (Rogero *et. al*, 2003). The extract obtained by immersion of sample in cell culture medium MEM (Eagle´s minimum medium) during 24 h was serially diluted and placed on cell cultured in a 96 wells microplate. The cytotoxicity effect was evaluated by measuring the neutral red uptake level by the optical density reading in Sunrise of Tecan spectrophotometer, at 540 nm. The cell viability percentage was calculated in relation to cell control in the assay (100% viability). HDPE were used as negative control and natural rubber latex was used as positive control.

**Resveratrol stability**

**Sample Preparation**

Resveratrol aqueous solutions were prepared in a concentration of 0.1 mg $mL^{-1}$ in three diluents: ethanol/water (1:1, v/v); methanol/water (1:1, v/v) and *tert*-Butyl alcohol/water (1:1, v/v).

To verify the resveratrol stability each prepared solution was submitted to irradiation doses of 0.0, 0.5 and 1.0 kGy using a Gammacell 220 source (Atomic Energy of Canada Limited, Ottawa Canada) with a dose rate of 2.41 kGy $h^{-1}$.

The resveratrol integrity was analyzed by high performance liquid chromatography (HPLC) utilizing no irradiated aliquots of each solution as control.

**HPLC analysis**

The HPLC system consisted of an ÄKTApurifier - GE equipped with a P-9000 quaternary pump and controlled with the UNICORN Manager 5.11. Quantifications

were performed on a $C_{18}$ Vydak column, 250 x 1 mm, 5-µm particle size, from Grace Davison Discovery Sciences.

The solvents used for the analysis were as follows: solvent A - 2% acetic acid:phosphate buffer (1:6, v/v); solvent B - pure acetonitrile. The linear gradient solvent system was delivered according to the following program: 0 to 6 min = 100% solvent A; from 6 to 18 min = gradient 0 to 100% solvent B, at a flow rate of 80 µL $min^{-1}$.

The injected sample volume was 80 µL and the chromatograms were recorded at 307 nm using a UV detector.

## RESULTS AND DISCUSSION

In the swelling assay the PVP hydrogel samples were characterized as their capacity of absorption of water and the obtained swelling curve is presented in Fig. 1. The swelling capacity was near to 1600% after 24 hours and reached the equilibrium after 6 hours of assay.

The results are comparable with a similar study by Ajji et al using hydrogel matrices composed of PVP in different concentrations, PEG and agar. The matrix with 5% of PVP and irradiated at 25kGy dose presented a swelling percentage of hydrogel dressing about 1500% after 24 hours (Ajji *et. al,* 2005). These results are very similar with the results achieved in this work, 1600%. Different irradiation doses could modify the crosslink density and consequently the swelling capacity.

Insert Fig. 1

The results of the gel fraction assay are presented in Table 2, where can be observed the result of 90%, indicating high crosslinking degree. The result presented in

the similar study with 6% PVP irradiated at 25kGy dose was about 81%, very similar to the achieved in this work. (Ajji *et. al*, 2005).

Insert Table 1

In the cytotoxicity assay the obtained curves of cellular viability are shown in Fig. 2. We can observe that the PVP hydrogel showed similar behavior of the negative control, as no cytotoxic. The positive control showed cytotoxic effect, presenting $IC_{50\%}$ about 72.

Insert Fig. 2

A preliminary study to analyze the resveratrol stability was performed to optimize the chromatographic conditions to get better results within a short time analysis and the analytical procedure described by Bader *et al*. (2007) was used as a reference. However, several parameters were modified and the results are presented in Table 2.

Insert Table 2

HPLC chromatograms of all resveratrol solutions presented the same behavior, showing only one peak for each sample with similar retention times and area percentages in different irradiation doses, as presented in Fig. 3.

Insert Fig. 3

In this studied conditions and range of irradiation doses was not detected formation of products and structural decomposition of resveratrol. These results differ from that obtained by Bader *et al*. noted that aqueous solutions of resveratrol saturated with pure gases (argon, $N_2O$ and air) presented decomposition of $2x10^{-5}$ mol $L^{-1}$ (Bader *et al*. 2007).

## CONCLUSION

In the studied conditions and range of irradiation doses the results of resveratrol stability suggest that resveratrol showed no structural decomposition by the primary and secondary radicals of water radiolysis.

The polymeric matrix composed by PVP, PEG and Agar showed appropriate physical and chemical characteristics to resveratrol immobilization and compose a hydrogel dressing for dermatological use.

The study might be continued with resveratrol incorporation in PVP hidrogel matrix before crosslinking irradiation.

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**Table 1.** Results of PVP hydrogel gel fraction assay.

| Matrix | $w_i$ | $w_e$ | % gel fraction |
|---|---|---|---|
| PVP hydrogel | 0.2167 | 0.1945 | 90 ± 0.6 |
| | 0.2379 | 0.2147 | |
| | 0.2623 | 0.2387 | |

**Table 2.** Retention time and areas of different resveratrol solutions exposed to 0.0, 0.5 and 1.0 kGy doses in a single fraction.

| Solvent | Dose (kGy) | Retention Time (min) | Area (%) |
|---|---|---|---|
| **Etanol 50%** | 0 | 4.250 | 99.60 |
| | 0.5 | 4.125 | 99.86 |
| | 1.0 | 4.125 | 99.76 |
| **Metanol 50%** | 0 | 4.250 | 99.40 |
| | 0.5 | 4.375 | 99.77 |
| | 1.0 | 4.375 | 99.47 |
| ***Tert*-Butyl alcohol 50%** | 0 | 4.125 | 98.16 |
| | 0.5 | 4.125 | 99.55 |
| | 1.0 | 4.250 | 99.76 |

**Figure 1.** PVP hydrogel matrix swelling curve.

**Figure 2.** Cell viability curves in the PVP hydrogel cytotoxicity test.

**Figure 3.** HPLC Chromatogram of resveratrol solutions irradiated at 0.0, 0.5 and 1.0 kGy doses.

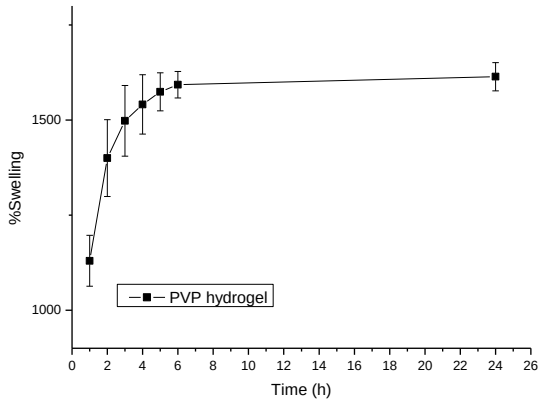


**Figure 1**

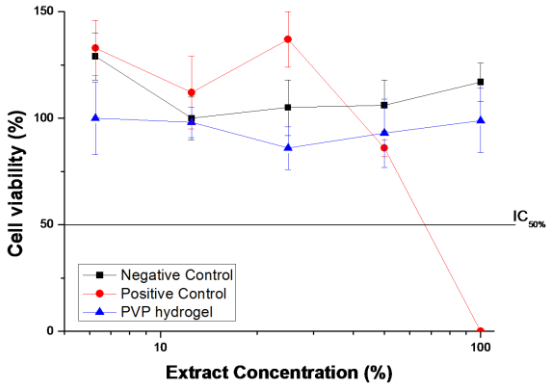


**Figure 2**

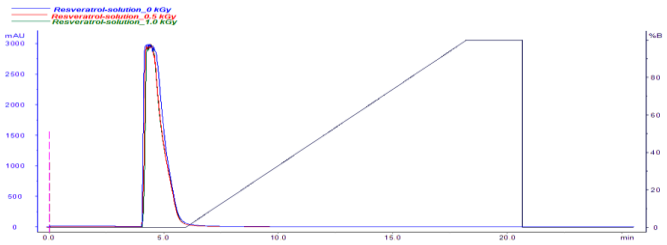


**Figure 3**