Radiation Physics and Chemistry 79 (2010) 283–285

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Radiation stability of resveratrol in immobilization on poly vinyl pyrrolidone hydrogel dressing for dermatological use

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ARTICLE INFO

Keywords:
Trans-resveratrol
PVP hydrogel
HPLC

ABSTRACT

The polyphenol trans-resveratrol is a natural phytoalexin, which is found in red wine and in a wide variety of plant species. Resveratrol displays a wide array of biological activities, such as modulation of lipid metabolism, anti-inflammatory and antioxidant activities. This active compound immobilized in polyvinylpyrrolidone (PVP) hydrogel could be very interesting for topical administration, as a dressing form for dermatological use. However, PVP hydrogel obtained by radiation-induced crosslinking 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. The integrity of these samples was compared to unirradiated resveratrol by HPLC. The PVP hydrogel matrix was characterized by gel fraction, swelling and *in vitro* biocompatibility test. The results of gel fraction and swelling degree were approximately 90% and 1600%, respectively. The cytotoxicity assay showed absence of toxicity for this formulation after crosslinking and sterilization, indicating that the PVP hydrogel formulation was appropriate for resveratrol immobilization to produce a dressing for dermatological use.

1. Introduction

The polyphenol trans-resveratrol (*trans*-3,4′,5-trihydroxystilbene) is a natural phytoalexin synthesized in response to infection or injury by a wide range of plants, including *Vitis vinifera* and a variety of medicinal plants. This compound was shown to possess exceptional activities and important biological, pharmacological and medicinal properties such as inhibition of lipid peroxidation and platelet aggregation, vasorelaxation, as well as anti-inflammatory and antioxidant activities (Iacopini et al., 2008; Frémont, 2000).

In vitro experiments indicate that resveratrol shows a very strong antitumor action on cells exposed to γ-rays (Bader and Getoff, 2006). The studies were performed in aqueous media saturated with air (acting free radicals: 46% OH, 54% O_2^-) or N_2O (90% OH, 10% H) as well as in airfree media (44% e_{aq}^-, 10% H, 46% OH). Under these experimental conditions various radiolytic products of resveratrol were determined by HPLC (Bader et al., 2008).

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 scavenger, preventing skin aging (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 demonstrates advantages in comparison to chemical reactions but can cause undesirable hydrolysis reactions, modifying the immobilized compound activity (Peppas, 1996).

Polyvinylpyrrolidone (PVP) has been used successfully as the basis for hydrogels manufacturing. These types of hydrogel can be used as polymeric matrix in a drug delivery system due to their biocompatibility, swelling capacity, ability to disperse different active compounds and control of solute transport (Ajji et al., 2005; Geever et al., 2008).

Our objectives were to establish the irradiated resveratrol stability during the hydrogel production process, i.e., simultaneous radio-induced crosslinking and sterilization and to develop the immobilization of resveratrol in a PVP hydrogel matrix to obtain a dressing for dermatological use.

The PVP matrix was characterized by swelling, gel fraction and *in vitro* test of biocompatibility by neutral red uptake cytotoxicity assay.

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doi:10.1016/j.radphyschem.2009.08.039

2. Materials and methods

The reagents used in this work were: 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.

2.1. Polymeric matrix preparation

The polymeric matrix was composed of 6% PVP K90, 1.5% PEG 300 and 0.5% agar. The membranes were obtained after irradiation of hydrogel in a circular mold, heat sealed and irradiated in a ^{60}Co gamma ray source with a 10 kGy h^{-1} dose rate and a 20 kGy dose.

2.2. Swelling assay

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

$$\text{swelling}\ (\%) = \frac{w_s - w_i}{w_i} \times 100 \quad (1)$$

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

2.3. Gel fraction

The gel fraction assay was performed in triplicate with dried samples. The extraction of the soluble fraction was accomplished with distilled water in a Soxhlet extractor during 40 h. After this period, the samples were dried until constant weight was reached. Gel fraction was calculated by:

$$\text{Gel fraction}(\%) = \frac{w_e}{w_i} \times 100 \quad (2)$$

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

2.4. 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 document 10 993-5, 1992) and the previously described methodology by Rogero et al. (2003). The extract obtained by sample immersion in cell culture medium MEM (Eagle's minimum medium) during 24 h was serially diluted and placed on cell cultured in a 96 well microplates. The cytotoxicity effect was evaluated by measuring the neutral red uptake level by the optical density reading with a Tecan Sunrise spectrophotometer, at 540 nm. The cell viability percentage was calculated in relation to the control cells in the assay (100% viability). HDPE and natural rubber latex were used as negative and positive controls respectively, both being treated in the same way as the hydrogel samples in the assay.

2.5. Resveratrol stabilities

2.5.1. Sample preparation

Resveratrol aqueous solutions were prepared at 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 solution prepared was submitted to irradiation doses of 0.0, 0.5 or 1.0 kGy in a Gammacell 220 source (Atomic Energy of Canada Limited, Ottawa Canada) with a dose rate of 2.41 kGy h^{-1} without degassing.

The resveratrol integrity was analyzed by high performance liquid chromatography (HPLC) using individual unirradiated controls for each solution.

2.5.2. HPLC analysis

The HPLC system consisted of an ÄKTApurifier-GE equipped with a P-9000 quaternary pump controlled by the UNICORN Manager 5.11 software. Quantifications were performed on a C_{18} Vydak column, 250 × 1 mm, 5-μm particle size, from Grace Davison Discovery Sciences.

The used solvents were as follows: solvent A = 2% acetic acid:phosphate buffer (1:6, v/v) pH 6.0; solvent B = pure acetonitrile. The linear gradient solvent system was delivered according to the following program: 0–6 min = 100% solvent A; from 6 to 18 min = gradient from 0 to 100% solvent B, with 80 μL min^{-1} flow rate.

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

3. Results and discussion

Gel fraction results are presented in Table 1. A crosslinking level of 90% was obtained, i.e., a fully crosslinked network. The result presented in a similar study by Ajji et al. with 6% PVP irradiated at 25 kGy was of about 81%, which is in agreement with our data (Ajji et al., 2005).

The swelling capacity for the PVP hydrogel was near to 1600% after 24 h and the equilibrium was reached after 6 h as presented in Fig. 1.

These results are similar to those obtained by Ajji et al. in the study of hydrogel matrices composed by PVP in different concentrations, PEG and agar. The matrix with 5% PVP and irradiated at 25kGy dose showed a swelling degree of about 1500% after 24 h (Ajji et al., 2005). These results are very similar to ours, 1600%. The gel fraction and swelling degree demonstrate that this is a highly crosslinked system and as it shows a high level of swelling in PBS solution, this system is suitable for the controlled release of drugs.

In the cytotoxicity assay, the percentages were plotted against extract concentration, resulting in cellular viability curves shown in Fig. 2. In this plot the sample with curves above 50% cell viability (cytotoxicity index line = $IC_{50\%}$) are considered noncytotoxic and those below or crossing the $IC_{50\%}$ line are considered cytotoxic. We can observe that PVP hydrogel behaved similarly to the negative control with a cell viability curve above the $IC_{50\%}$. Therefore it is considered safe (or noncytotoxic). The positive control showed cytotoxic effect, presenting an $IC_{50\%}$ of about 72.

The analytical procedure described by Bader et al. (2008) for chromatographic conditions optimization to achieve better results within a shorter time analysis was used as a reference in order to analyze the resveratrol stability. However, several parameters were modified and the results are presented in Table 2.

Table 1
Results of PVP hydrogel gel fraction assay.

Matrix	w_i (g)	w_e (g)	% gel fraction	Medium (%) ± sd
PVP hydrogel	0.2167	0.1945	89.8	90.3 ± 0.6
	0.2379	0.2147	90.2	
	0.2623	0.2387	91.0	

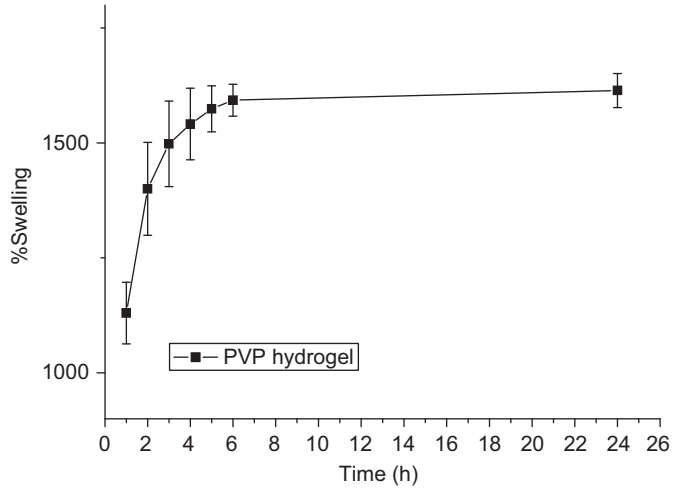

Fig. 1. PVP hydrogel matrix swelling curve.

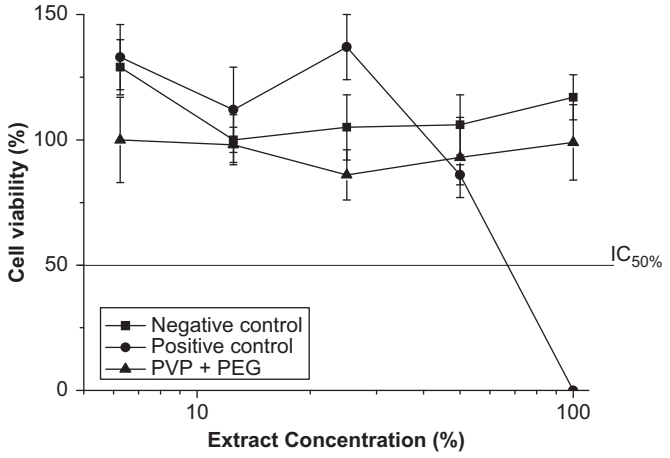

Fig. 2. Cell viability curves in the PVP hydrogel cytotoxicity test.

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 (%)
Ethanol 50%	0	4.250	99.60
	0.5	4.125	99.86
	1.0	4.125	99.76
Methanol 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

All resveratrol solutions revealed the same HPLC chromatograms, showing only one peak for each sample with similar retention times and areas percentage with different irradiation doses, as shown in Fig. 3.

In the studied conditions and range of irradiation doses, we could not detect formation of byproducts resulting from structural decomposition of resveratrol. These results differ from those obtained by Bader et al. where they noted that resveratrol aqueous solutions saturated with pure gases (argon, N_2O and

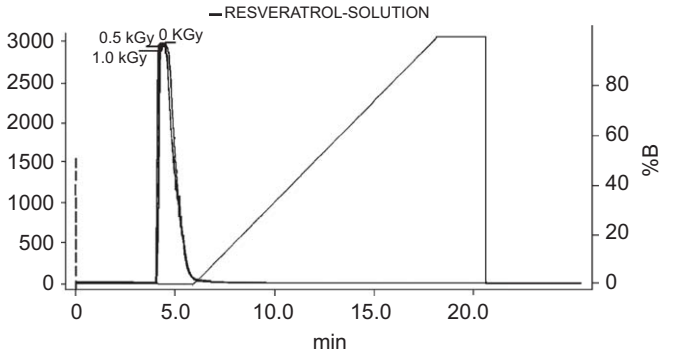

Fig. 3. HPLC chromatogram of resveratrol solutions irradiated at 0.0, 0.5 and 1.0 kGy doses.

air) showed 2×10^{-5} mol L^{-1} resveratrol decomposition (Bader et al., 2008).

However, our experiment differs from Bader et al. in the solvent type. We used water–alcohol mixtures instead of water. Actually, no degradation products were observed by HPLC. A possible explanation could be that the free radicals originating from water radiolysis are scavenged by the corresponding alcohol used.

4. 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 suitable physical and chemical characteristics to resveratrol immobilization to compose a hydrogel dressing for dermatological use.

The study might be continued with resveratrol incorporation in PVP hydrogel before irradiation.

Acknowledgements

The authors wish to thank Embrarad and CTR-IPEN (Elizabeth Somessari and Carlos Gaia da Silveira) for the irradiation process and the student Rezolina Pereira dos Santos (IAL) for microplate cell culture preparations.

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