Gamma radiation-induced synthesis and characterization of Polyvinylpyrrolidone nanogels

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Abstract. Due to the importance of bioactive peptides, proteins and drug for pharmaceutical purpose, there is a growing interest for suitable delivery systems, able to increase their bioavailability and to target them to the desired location. Some of the most studied delivery systems involve encapsulation or entrapment of drugs into biocompatible polymeric devices. A multitude of techniques have been described for the synthesis of nanomaterials from polymers, however, the use of ionizing radiation (γ, e-), to obtain nano- and microgels polymer is characterized by the possibility of obtaining products with a high degree of purity. Although, in the world, electronic radiation is used for this purpose, gamma radiation has not been utilized for these purposes. In this paper is developed the formulation of Polyvinylpyrrolidone (PVP) nanogels synthesized by gamma radiation techniques, for their evaluation as potential system of drug delivery. Experiments were performed in absence of oxygen using aqueous solutions of PVP (0.05% -1%). Crosslinking reactions were carried out at 25 °C in a gamma irradiation chamber with a 60Co source (MPX-γ 30). The Viscosimetry, Light Scattering, X-Ray Diffraction and Transmission Electron Microscopy (TEM), were used as characterization techniques.

1. Introduction
In the recent years, an important factor stimulating research on radiation-induced reactions of polymers in aqueous solution is the application of this technique for synthesizing new materials or modifying the properties of polymers [1], a significant advantage of the use of radiation is that the processes can be considered “solvent free” respect to conventional synthesis procedures thereby reducing or avoiding the presence of additives that could be potentially harmful. The use of radiation has involved, as might be expected, the field of nanotechnologies in the production of new materials and structures that have both fundamental interest and potential applications in areas such as biomedical sciences, electronics, optics, and material sciences [2, 3]. In this paper is develop the synthesis of Polyvinlylpyrrolidone (PVP) nanogels by ionizing radiation techniques (gamma radiation), for their evaluation as potential system of drug delivery.
Polymeric micro and nanogels are probably the most prominent examples of waterborne products obtained by radiation technology. They have a broad field of actual and potential applications ranging from filler materials in coating industry to modern biomaterials [3, 4]. Nanogels are promising novel pharmaceutical carriers for small biologically active agents and biomacromolecules. The advantages of these systems include simplicity formulation with the drugs, high loading capacity, and stability of the resulting formulation in dispersion. These systems allow immobilization of biologically active compounds of diverse structure including charge drugs, low molecular mass hydrophobes, and biopolymers. Furthermore, nanogels can be chemically modified to incorporate various ligands for targeted drug delivery. The in vivo and vitro studies suggest that nanogels can be used for efficient delivery of biopharmaceuticals in cell as well for increasing drug delivery across cellular barriers [5-7].

A multitude of techniques have been described for the synthesis of polymeric micro and nanogels, however, the use of ionizing radiation ($\gamma$, e$^-$), to obtain nano- and polymer microgels, is characterized by the possibility of obtaining products with a high degree of purity [5]. The main factors that contribute to the success of these technologies are: water as a human and environment-friendly solvent, lack of any potentially harmful chemicals (monomers, initiators, crosslinking agents, etc.), simple production schemes, parallel synthesis and sterilization of the products in the sealed, final packages, valuable medical properties of the products, broad range of applications, and the possibility of manufacturing “smart” gels reacting to temperature, pH, light, etc [8-10]. Particulars, the polyvinylpyrrolidone nanogels can be obtained by gamma radiation, based not on polymerization, but on intramolecular crosslinking of polymers chains, in aqueous solutions. The nanogel characterization was performance by Viscosimetry, Light Scattering, X-Ray Diffraction and Transmission Electron Microscopy (TEM). The results showed that in dependence on the polymer concentration and the dose rate two different crosslinking reactions can be obtained. Irradiation experiments at room temperature in diluted solution with further increasing of the radiation dose lead to the formation of PVP nanogels due to an intramolecular crosslinking reaction. By using both light scattering and TEM the PVP nanogels were measured, these showed a size distribution of 50.10 nm. They have lots of advantages over conventional systems since they enhance the delivery, extend the bioactivity of the drug by protecting them from environmental effects in biological media, show minimal side effects, demonstrate high performance characteristics, and are more economical since minimum amount of expensive drugs are used.

2. Experimental

2.1 Chemicals and materials. The chemicals used in this study were mostly reagent with high analytical grade and used as received (Sigma-Aldrich) without further treatment. Polyvinylpyrrolidone-PVP, type K-90 (> 99.99). All chemical solutions were prepared with water purified by distillation and in order to remove any dust particles and/or polymer aggregates, solutions were filtered subsequently through filters of 0.45 and 0.22 $\mu$m pore size. Gamma irradiation were carried out with a panorama $^{60}$Co source at 15 kGy dose.

2.2 Synthesis of PVP nanogels. The synthesis was carried out with a panorama $^{60}$Co source of Hungarian manufacturing and a homogeneity 1.3 at room temperature. The irradiation process was monitored by Fricke dosimetry and film dosimeters using Perpex. Irradiation times were determined by calculation CaliPMMA platform, developed at the Laboratory of High Dose Dosimetry Centre for Technological Applications and Nuclear Development (CEADEN) [11]. Previously the samples were collected in glass volumetric -100 and 50mL- and saturated for 10 minutes under argon (Figure 1).

![Scheme of experimental installation.](image-url)
2.3 Characterization.
- The viscosimetric study of polymeric solutions was conducted both before and after being irradiated, according to the method described in ISO 1628-1: 1998 (E) [10] and the Cuban Norm NC 30-13 [11], using a capillary viscometer type Ubbelhode, equipped with a thermostatic bath controlled by a water recirculating at 25.0 ± 0.01 °C and a timer. From the results obtained, the viscosimetric parameters shown in Table 1 are calculated.

| Common name       | Symbol and mathematical definition                                      |
|-------------------|-------------------------------------------------------------------------|
| Relative viscosity| $\eta_r = \eta / \eta_0 = t / t_0$                                      |
| Specific viscosity| $\eta_{sp} = \eta_r - 1 = (\eta - \eta_0) / \eta_0 \approx (t - t_0) / t_0$ |
| Reduced viscosity  | $\eta_{red} = \eta_{sp} / C$                                            |
| Inherent viscosity | $\eta_{inh} = (\ln \eta_r) / C$                                        |
| Intrinsic viscosity| $[\eta] = (\eta_{sp} / C)_{c=0} = [(\ln \eta_{red}) / C]_{c=0}$        |

- The average and distribution size of particle of irradiated solutions was measured by dynamic light scattering (DLS, by its acronym), using a particle analyser, DelsaTMNano.

- X-ray diffraction (XRD) powder patterns of sample was recorded with a Bruker D8 Advance Diffractometer and CuKα radiation (1.54183 Å).

- The morphology and particle size distribution of the produced powders were investigated by a high-resolution electron microscopy (JEOL JEM 2010), operated with an accelerating voltage of 200kV, a resolution of 1 Angstrom and an Orius camera SC200D 2K x 2K, 15 square per second incorporated. Samples for TEM were prepared by letting a drop of the samples dispersed in ethanol evaporate on top of a carbon-coated copper grid of 200 meshes.

3. Results and Discussion.

3.1 Viscosimetric study.
When comparing the values of the reduced viscosity of the solutions to 0.1% PVP unirradiated and irradiated to 10 kGy, it can be seen a considerable decrease of this magnitude, indicating the possible formation of Nanogels. Because, as nanogels particles are more compact than the spiral form of its linear polymer, the viscosity of the nanogels is smaller than linear macromolecules at the same concentration.
Table 2. Viscosimetric parameters determination before and after irradiation for PVP (0.01 %) solution.

| Concentration of PVP (g/mL) | 0.001 (Before irradiation) | 0.001 (After irradiation) |
|-----------------------------|-----------------------------|----------------------------|
| Relative viscosity          | 1.11                        | 1.02                       |
| Specific viscosity          | 0.11                        | 0.02                       |
| Reduse viscosity            | 114.21                      | 23.35                      |
| Inherent viscosity          | 108.14                      | 23.09                      |

4. Analysis of light scattering.

- Hydrodynamic Radius.
The hydrodynamic radius of the PVP prior to irradiating is 25.26 nm and after irradiation of PVP (0.1 %) solution to 15 kGy this parameter low, at 18.49 nm. This diminution is an indicator of the process of coil shrinkage resulting from intramolecular crosslinking and nanogels are formed [12].

- Analysis of average molecular weight Mw.
The value of the average molecular weight for the solution of PVP -before and after irradiation- were obtained by DLS using as input parameters $\alpha = 0.55$ and $\beta = 0.000565$ [11]. The value of molecular weight for PVP (K-90) unirradiated reported is 1200000 Da [13], the solution after irradiating had a molecular weight of $3.48 E + 06$ Da. After irradiating of solution under study, a small increase in molecular weight is obtained, despite the decrease in reduced viscosity. Therefore, is logical to think that there is a few contribution of intermolecular recombination in spite of the used conditions. This effect has been studied in nanogel formation for some authors [14, 15].

4.1 X-Ray Diffraction.

Figure 2 shows the diffractogram obtained for the solid sample of PVP with 0.1% concentration and irradiated at 15 kGy. No specific signal is observed by diffraction, which shows its amorphous nature, because it is a polymer, although a slight tendency to nano-crystallinity appears. Said result shows that both the irradiation and the polymer concentration used for obtaining these nanogels do not affect as such during initial material.

![Figure 2](image.png)

**Figure 2.** XRD pattern of powders obtained for solution of PVP (0.1 %) and doses (15 kGy).

4.2 Transmission Electron Microscopy (TEM).
The TEM image of solution of PVP (0.1 %) concentration, and an irradiation dose of 15 kGy is shown in Figure 3. This image suggests that these conditions of concentration and dose, allows Nanogels formation mainly in elliptical shape, which are slightly agglomerated. The histogram in Figure 3 was obtained, based on a count of 100 particles from several images, to determine the particle size distribution, which was adjusted to a lognormal distribution. The resulting average diameter was 50.10 nm, the sample has a narrow size distribution given by a dispersion value of 1.04. This evidences a considerable uniform particle size.

Conclusion
PVP Nanogel particles were synthesized using gamma radiation, based not on polymerization, but on intramolecular crosslinking of polymers chains, in aqueous solutions. Although the concentration used in nanogel formation is below its overlap concentration, there is some contribution of intermolecular recombination in the obtained product, it is evidenced in the increase of molecular weight. The synthesized nanogels are practically amorphous because they tend at agglomerated in solid state. Their morphology is mainly elliptical forming nanoparticles in solution, for studied concentration at 15kGy. Since their properties as biomaterial the nanogel could be used for pharmaceutical or environmental purpose.

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