

Synthesis of poly(N-vinyl pyrrolidone) (PVP) nanogels by gamma irradiation using different saturation atmospheres

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ABSTRACT

Nanogels are internally crosslinked particles of nanometric size used in various fields e.g. as such as carriers in drug delivery systems. They can be produced using ionizing radiation in dilute aqueous solutions. This method is carried out in a pure polymer-solvent system, avoiding the addition of any additives such as monomers, surfactants, catalysts and crosslinking agents and no further purification step is necessary. Poly(N-vinyl pyrrolidone) (PVP K-90) nanogels were prepared by gamma irradiation in an aqueous solution. The samples were prepared in triplicate in multipurpose cobalt-60 gamma irradiator using 1, 10, 25 and 100 mM PVP solutions. Samples were irradiated in argon and nitrous oxide conditions with doses from 1 kGy up to 25 kGy with 10 kGy/h dose rate. The mean particle size (R_h) was determined by Dynamic Light Scattering (DLS) and radius of gyration (R_g) and weight-average molecular weight (M_w) by Static Light Scattering (SLS). These samples were morphologically characterized using Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM). Samples prepared with 100 mM PVP K-90 solution formed macroscopic gels, in the samples obtained with 25 mM PVP K-90 solution there was a prevalence of intermolecular crosslinking. On the other hand, in the samples generated with 10 mM PVP K-90 solution, there was a predominance of intramolecular crosslinking demonstrated in the tendency to: decrease in the radius of gyration (R_g), in the constancy of the weight-average molecular weight (M_w), in the increase in polymer coil density (ρ_{coil}), in the R_g/R_h ratio (shape factor) around 1.0 indicating homogenous, internally cross-linked spheres, in the high relief spherical structures observed in the AFM images and in the spherical particles with high contrast observed in the TEM images. The saturation of the samples with nitrous oxide doubled formation of hydroxyl radicals, favoring the generation of polymeric radicals. Higher average number of radicals in each macromolecule contributed to the higher number of intramolecular crosslinks.

1. Introduction

Nanoscience studies molecules and structures on a nanometric scale ranging from 1 to 100 nm and nanotechnology applies this science in a practical way, converting its theory into useful applications. This technology has advanced considerably in recent years and has become of fundamental importance in the biomedical field with the development of drug delivery systems, imaging probes and diagnostic biosensors. Nanocarriers have stood out in this scenario, acting as drug carriers, platforms for combinational therapy, multifunctional diagnosis and

theranostics (Bayda et al., 2020; Yin et al., 2020).

Nanogels are internally crosslinked particles of nanometric size used in various fields e.g. in drug delivery. There are at least two definitions for nanogels, one is based on the definition of polymeric gel, that is, a two-component system formed by a permanent three-dimensional network of polymer chains and the solvent within this network, however, presenting nanometric size with variation from 1 to 100 nm (IUPAC Compend. Chem. Terminol., 2019). Another definition characterizes nanogel as a single internally crosslinked polymer chain forming separate macromolecule (Ulański et al., 1998; Ulański and Rosiak, 1999;

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Matusiak et al., 2020; Yin et al., 2020).

One of the first appearances of the term nanogel was in the work of Graham and Cameron in 1998 (Graham and Cameron, 1998). Later on nanogels started to be used in number of different applications e.g. as a drug carrier developed for the delivery of antisense oligonucleotides, consisting of crosslinked networks of water-soluble nonionic polymer (PEG) and cationic polymer, polyethyleneimine (PEI) (Vinogradov et al., 1999). Thus, the word nanogel was used to describe hydrogel particles on a nanometric scale capable of swelling in an aqueous environment and consisting of crosslinked polymers. These nanocarriers exhibit, under different environmental conditions, the shrinkage-swelling properties characteristic of hydrogels and the highly hydrated nature. Additionally, they enable the encapsulation of hydrophilic or hydrophobic drugs in their internal network due to their 3D structure, protecting the drugs from degradation. Nanogels have large surface area, stable internal network, stability and very low solution viscosity (Ahmed et al., 2020; Sütėkin and Güven, 2019; Yin et al., 2020).

In addition to carrying drugs, nanogels also act as a carrier system for vaccines, contrast agents, chemical and biological sensors, super-absorbers, nanoreactors, nanodevices, among others (Sütėkin and Güven, 2019). They can be prepared using a wide variety of synthetic and natural polymers and have different structures like core shell nanoparticles, hollow nanogels, yolk shell and double walled structure (Ahmed et al., 2020). Targeting ligands such as peptides, antibodies or carbohydrates can be conjugated to functional groups present in nanogels, as well as (bio)molecular drugs and oligonucleotides, such as antisense RNA, antagomiRs or microRNA. Crystallization, ionic crosslinking, radiation crosslinking, crosslinking polymerization and functional group crosslinking are different crosslinking methods used in the preparation of nanogels (Ahmed et al., 2020; Ditta et al., 2019; Sütėkin and Güven, 2019).

Rosiak and coworkers proposed an alternative method of initiating intramolecular crosslinking through the use of ionizing radiation in dilute aqueous solutions. This method is carried out in a pure polymer-solvent system, avoiding the addition of any additives such as monomers, surfactants, catalysts or crosslinking agents. This is a considerable advantage, especially for the development of products for biomedical applications because there is no need for a purification step. This method is reproducible, versatile and cost effective, however, it has as a disadvantage the limitation of applicability on a large scale, since methods involving high energy radiation are not readily implemented on existing production lines (de Lima et al., 2020; Sütėkin and Güven, 2019; Kadłubowski, 2014; Matusiak et al., 2018; Ulański et al., 1998; Ulanski and Rosiak, 1999).

Irradiation of deoxygenated dilute aqueous solutions of water-soluble polymers with gamma rays or high energy electrons promotes the formation of nanogels. In this method, most of the radiation energy is absorbed by water, generating short-lived reactive species such as OH radicals, hydrated electrons and H-atoms. Electrons are inert relative to simple, aliphatic water-soluble polymers and when the solution is saturated with N₂O they can be converted into additional OH radicals. Hydrogen atoms and hydroxyl radicals abstract hydrogen atoms from macromolecules, producing polymer radicals. These radicals can react in different reactions of one radical such as H atom shift and degradation (chain scission) and different reactions of two radicals such as intermolecular crosslinking, intramolecular crosslinking, intermolecular disproportionation and intramolecular disproportionation (Ditta et al., 2019; Sütėkin and Güven, 2019; Kadłubowski, 2014).

Intramolecular crosslinking of polymeric radicals is the basic reaction for the formation of nanogels, however, there is a competition between the aforementioned reactions that depends on the irradiation conditions and the composition of the system (polymer concentration, dissolved gases, molecular weight, chemical structure, etc.). For nanogels to be formed, the polymer coils must be well separated from each other and a high numbers of radicals must be generated in a single coil. When the average number of radicals per chain is less than one, the

single radical can undergo rearrangements or scission or can recombine with a radical in a neighboring polymer chain, promoting the binding of macromolecules with an increase in the average molecular weight, forming a macroscopic hydrogel. In contrast, when the number of radicals per chain exceeds one, nanogels are formed due to the intramolecular crosslinking domain. Very low polymer concentrations and high dose per electron pulse promote the formation of nanogels (Ulański et al., 1998; Ulanski and Rosiak, 1999; Ditta et al., 2019; Sütėkin and Güven, 2019; Kadłubowski, 2014).

Recently, Sütėkin and Güven, 2019, developed poly(N-vinyl pyrrolidone) (PVP) nanogels in the range of 30–250 nm from dilute aqueous solutions using gamma irradiation and electron beam. The authors investigated two dose rates in gamma irradiation, 0.8 kGy/h and 10 kGy/h with three different doses, 5, 10 and 15 kGy under nitrous oxide saturation (Sütėkin and Güven, 2019). The goal of our work was to develop PVP nanogels from dilute aqueous solutions using gamma irradiation under two different saturation atmospheres, nitrous oxide and argon, evaluating the influence of the gas on the formation of nanogels and performing a morphological study with different microscopy techniques to particle size analysis. Lower and higher doses than those used by Sütėkin & Güven, that is, 1, 2 and 25 kGy, were studied maintaining a high dose rate (10 kGy/h) which in the future may allow obtaining nanogels with high-yield. Another original contribution of this work is the study of the influence of saturation gas on the formation of nanogels.

The samples were prepared in triplicate in multipurpose cobalt-60 gamma irradiator using 1, 10, 25 and 100 mM PVP solutions. Samples were irradiated in argon and nitrous oxide conditions with doses from 1 kGy up to 25 kGy with 10 kGy/h dose rate. These samples, as well as the unirradiated PVP, were morphologically characterized using Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM). The mean particle size of the samples and the polydispersity index was analyzed by Dynamic Light Scattering (DLS) and the determination of radius of gyration and weight-average molecular weight was performed using Static Light Scattering (SLS). Coil density has been also calculated and influence of irradiation dose and PVP concentration on these parameters was also analyzed.

2. Experimental

2.1. Materials

Poly(N-vinyl pyrrolidone), Kollidon® 90 F (PVP K90), with weight-average molecular weight of $M_w = 1,000,000$ – $1,500,000$ g/mol (according to manufacturer information) and number-average molecular weight of $M_n = 360,000$ was purchased from BASF (Ludwigshafen, Germany) and used without further purification (Buhler, 2008). The solutions were prepared using Gehaka-Ultrapur filtered water with a maximum conductivity of 0.05 μ S/cm and saturated for 15 min with argon (99.99%, Linde) or with N₂O (99%, for atomic absorption, White Martins).

2.2. Irradiation by ⁶⁰Co source

Dilute aqueous solutions of PVP were prepared freshly by stirring overnight at room temperature. Four concentrations, 1, 10, 25 and 100 mM were produced in triplicate. These solutions were placed in 20 mL glass vials covered with Parafilm and saturated with argon or N₂O for 15 min before irradiation.

Irradiation with γ -rays was carried out at Nuclear and Energy Research Institute in São Paulo, Brazil, in Cobalt 60 Multipurpose Irradiator in stationary mode with samples stored statically to avoid dose rate variation and provide higher precision in dose administration. The irradiation process was divided into two steps, one with irradiation of the samples in the corresponding doses for half the time necessary for the irradiation and the other rotating the samples at an angle of 180°

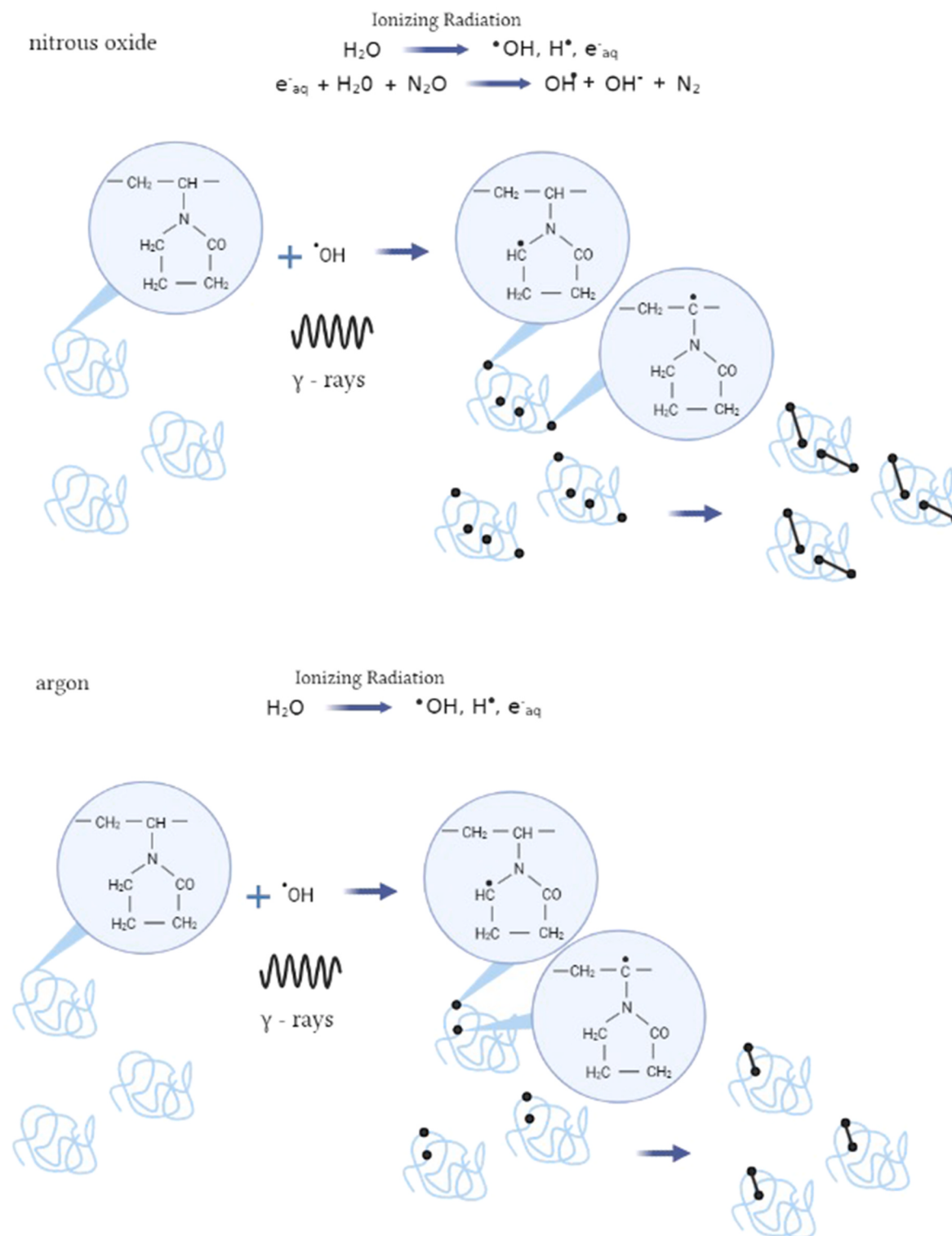


Fig. 1. Representation of radiation-induced synthesis of PVP nanogels in nitrous oxide and argon saturation (in nitrous oxide there is a higher yield of the polymer radical).

around their axis completing the remaining half of the samples irradiation time. A dose rate of 10 kGy/h at room temperature and five different irradiation doses (1, 2, 5, 10 and 25 kGy) for each concentration were used. The irradiation was performed in triplicate. The two-sided method used in the irradiation of the samples allows the calculation of DUR (Dose Uniformity Ratio) that approaches unity ($\text{DUR} < 1.05$)

for irradiation of research samples. Partial evaluations of dose rate was performed using Amber Perspex 3042 dyed-polymethylmethacrylate dosimeters (Harwell Dosimeters, UK) located on the front and back of the material are required.

2.3. Characterization methods

2.3.1. Dynamic Light Scattering (DLS) measurements

DLS technique was used to determine the hydrodynamic radius (R_h) of the irradiated samples and unirradiated PVP solution, as well as the polydispersity index to evaluate the particle size distribution. The analyses were performed in Zetasizer Nano ZS (Malvern Instruments Ltd., UK) equipment by Photon Correlation Spectroscopy at 25 °C, using ultrapure water as dispersant, cuvette DTS0012, equilibrium time of 120 s and the measurement angle was fixed to 13°. The samples and the solvent were filtered through a syringe filter with a pore size of 0.45 μm and 0.2 μm (Minisart, Sartorius), respectively, before the measurements and for each value obtained three measurements were performed.

2.3.2. Static Light Scattering (SLS) measurements

SLS measurements were taken to determine the weight-average molecular weight (M_w) and radius of gyration (R_g) of the irradiated samples and unirradiated PVP solution using Dawn® Heleos II – Multi Angle Light Scattering (MALS) equipment from Wyatt Technology Corporation ($\lambda = 658 \text{ nm}$) at 25.0 ± 0.1 °C with Zimm plot method. The intensity of the scattered light was measured at 18 different angles in the range of 22.5°–147° and the refractive index increment (dn/dc) used was 0.17 mL/g (Tumolo et al., 2004). Before the measurements, the samples and the solvent were filtered through a syringe filter with a pore size of 0.45 μm and 0.2 μm (Minisart, Sartorius), respectively.

2.3.3. Atomic Force Microscopy (AFM)

Irradiated samples and unirradiated PVP solution were diluted using ultrapure water and 10 μL of these dilutions were cast on silicon substrates (Ted Pella® 5 × 5 mm) and dried in a desiccator overnight. Multimode 8-HR Atomic Force Microscope (AFM) (Bruker) was used with ScanAsyst operating mode in Nanoscope 9.1 software to obtain the images at room temperature. The images were performed using silicon tip on nitride lever, model ScanAsyst-air (Bruker) having f_0 values of 70 kHz.

2.3.4. Transmission Electron Microscopy (TEM)

Irradiated samples and unirradiated PVP solution were diluted using ultrapure water and dripped onto a carbon-coated copper grid and dried in a desiccator overnight. Images were obtained using JEOL JEM-2100 F TEM-FEG microscope (JEOL, Tokyo, Japan).

3. Results and discussion

In the 1950s, Charlesby began studies involving the radiation chemistry of water-soluble polymers and since then several research groups have been dedicated to this topic. Rosiak and collaborators developed, in the late 1990s, a radiation method of nanogel synthesis based on intramolecular crosslinking of linear macromolecules (Charlesby and Alexander, 1955; Matusiak et al., 2020, 2018; Ulański et al., 1998).

The radiation-induced nanogel synthesis does not require the presence of monomers, initiators, crosslinking agents and surfactants. Free radicals are generated in a very simple system consisting only of an aqueous solution of a hydrophilic polymer. Water absorbs most of the radiation energy when a dilute aqueous solution of polymer is subjected to ionizing radiation in a process known as water radiolysis. As a result, hydrogen atoms, hydroxyl radicals and hydrated electrons are formed (Mozumder, 1999).

Hydrogen atoms and hydroxyl radicals abstract hydrogen atoms from macromolecules, generating polymer radicals called macroradicals. In the absence of oxygen macroradicals recombine with each other. Oxygen can react with macroradicals leading to a reaction pathway, which may include the formation of peroxides, ketones, aldehydes and carboxyl groups and finally may lead to chain scission. Therefore, the polymer solutions must be purged with an inert gas, for

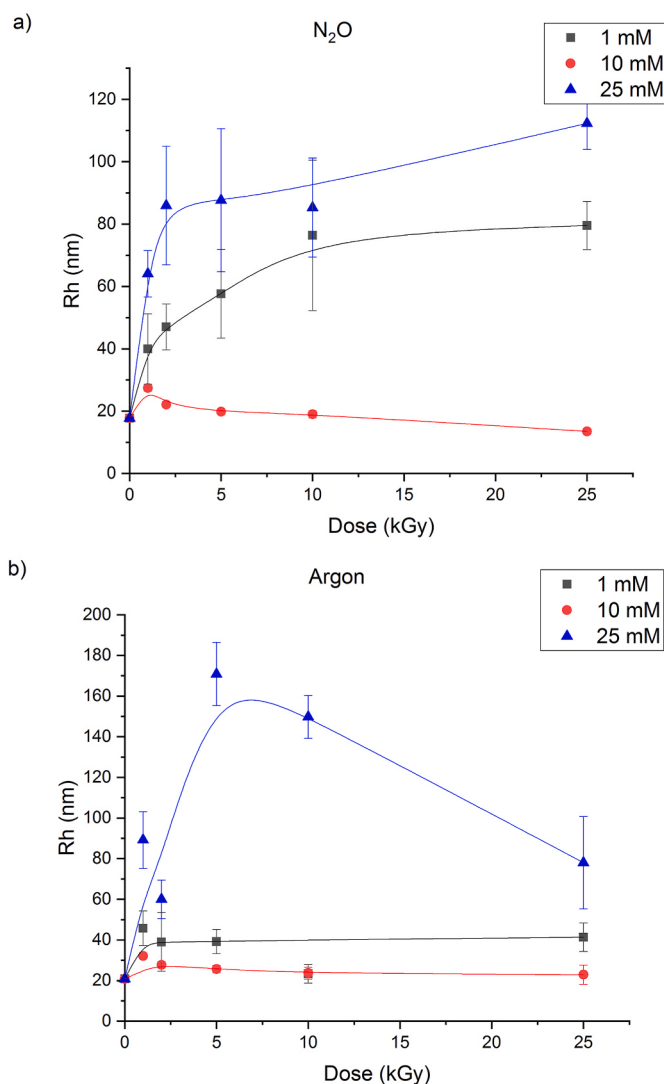


Fig. 2. Changes in hydrodynamic radius (R_h) of PVP as a function of absorbed dose under nitrous oxide and argon saturation. ■ [PVP] = 1 mM; ● [PVP] = 10 mM; ▲ [PVP] = 25 mM. Additional lines were added to guide the eye.

example argon. In order to increase the yield of the polymer radical, nitrous oxide can be used to saturate solutions. In this procedure hydrated electrons give rise to hydroxyl radicals, doubling the yield of the latter (Sonntag C. von, 1987).

The basic process in the radiation-induced synthesis of nanogels involves intramolecular crosslinking that occurs between two radicals at the same polymer chain, but in different polymer segments, forming a covalent intramolecular network. PVP, poly(N-vinyl pyrrolidone) when subjected to ionizing radiation in an oxygen-free dilute aqueous solution undergoes hydrogen abstraction predominantly in the methylene and methyldene groups (Davis et al., 1981; Dispenza et al., 2020). In Fig. 1 the radiation-induced synthesis of PVP nanogels in argon and nitrous oxide saturation is schematized.

The favoring of intramolecular crosslinking over other possible reactions (degradation, H atom shift, intermolecular crosslinking, intramolecular and intermolecular disproportionation) during irradiation of a dilute aqueous solution of PVP occurs under specific irradiation conditions. Since intramolecular crosslinking is based on the probability that one radical will combine with another in the same chain, it is necessary to generate the highest possible number of radicals at each chain and to properly separate the polymer coils. Coil separation can be achieved using low polymer concentrations and more effective

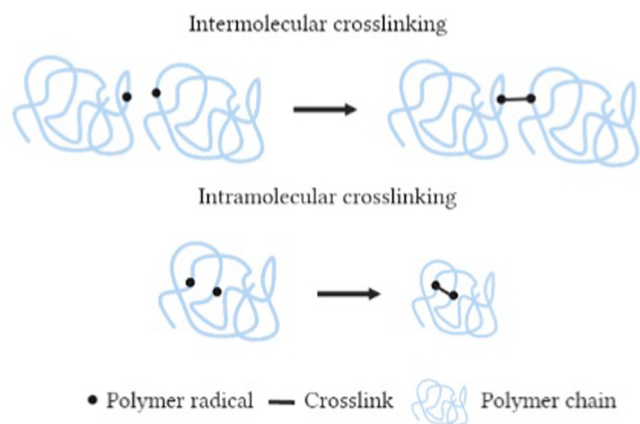


Fig. 3. - Recombination reactions in the absence of oxygen (based on Matusiak et al., 2020).

generation of radicals per chain can be achieved with high ionizing radiation dose rate. When the opposite occurs, that is, high polymer concentration and very low ionizing radiation dose rate promoting a low number of radicals per polymer chain, the formation of intermolecular crosslinking is favored, generating an intermolecular polymer network that characterizes macroscopic gels and wall-to-wall hydrogels (Sütekin and Güven, 2019; Kadlubowski, 2014; Matusiak et al., 2018).

During the generation of nanogels by radiation-induced synthesis, constant weight-average molecular weight and a significant decrease in the radius of gyration of macromolecules are observed. The decrease in the radius of gyration occurs due to the formation of internally cross-linked compact structures. An increase in coil density is also observed, indicating further evidence of intramolecular crosslinking (Kadlubowski, 2014; Matusiak et al., 2020).

This paper contributes to the advancement of studies related to radiation-induced synthesis of nanogels from an aqueous solution of PVP addressing different atmospheres of saturation, argon and nitrous oxide, in addition to different PVP concentrations and irradiation doses (An, 2009, 2010; An et al., 2011; Dispenza et al., 2012; Sütekin and Güven, 2019; Ghaffarlou et al., 2018; Kadlubowski et al., 2012).

3.1. Dynamic Light Scattering

The hydrodynamic radius (R_h) of the irradiated samples and unirradiated PVP solutions, all prepared in triplicate, are expressed in Fig. 2. Samples prepared with 100 mM PVP solution formed macroscopic gels and were not evaluated.

In Fig. 2 changes in average hydrodynamic radius (R_h) as a function of the applied irradiation dose for different concentrations of PVP under nitrous oxide and argon saturation are presented. Gamma irradiation of 25 mM PVP solution saturated with nitrous oxide resulted in an increase of R_h from 18 to 112 nm. This indicates a prevalence of intermolecular crosslinking since the concentration of PVP in this case (2.78 g/L) approaches the critical concentration of this polymer i.e. 4 g/L (Kadlubowski et al., 2012). The same behavior was observed in an argon atmosphere; however, there was a more expressive increase in the value of the hydrodynamic radius reaching around 170 nm. In an argon atmosphere, intermolecular recombination was favored, while in a nitrous oxide atmosphere, intramolecular crosslinking was favored due to the

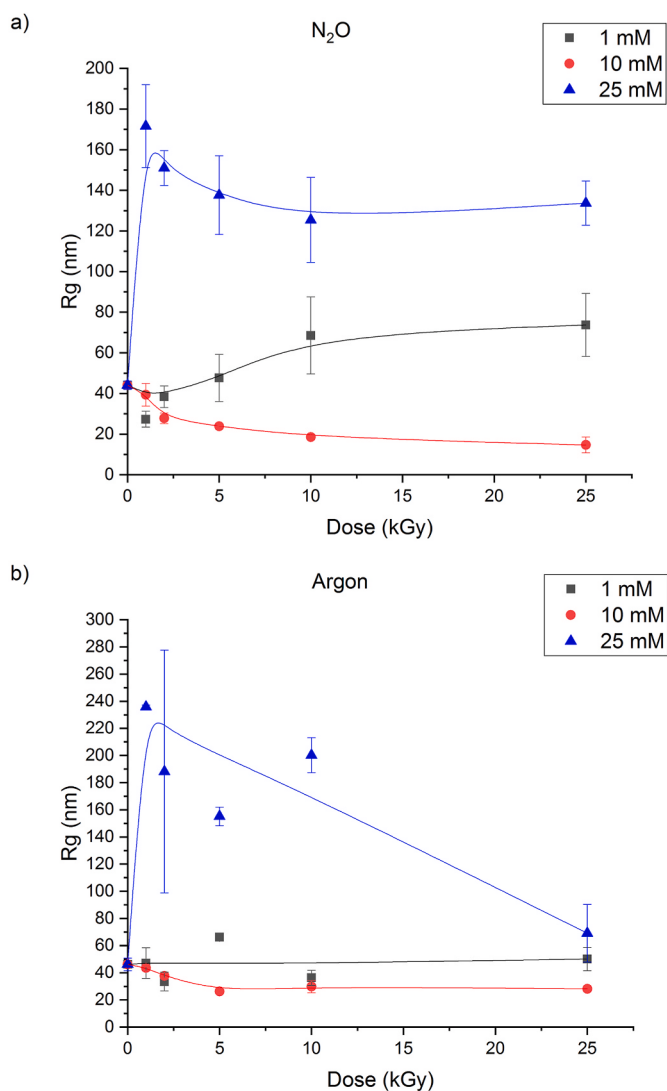
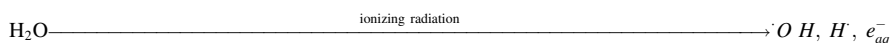


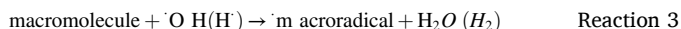
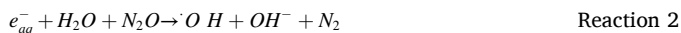
Fig. 4. Changes in radius of gyration (R_g) of PVP as a function of absorbed dose under nitrous oxide and argon saturation. ■ [PVP] = 1 mM; ● [PVP] = 10 mM; ▲ [PVP] = 25 mM. Additional lines were added to guide the eye.

higher number of radicals. Thus, the increase in hydrodynamic radius at N_2O saturation did not exceed 112 nm.

Water from a dilute aqueous solution of PVP K-90 absorbs most of the radiation energy when the solution is subjected to ionizing radiation forming short-lived reactive species predominantly OH radicals, hydrated electrons and H atoms as demonstrated in **Reaction 1**. In the presence of nitrous oxide, hydrated electrons are converted into hydroxyl radicals, doubling their yield in the medium as shown in **Reaction 2**. Polymeric radicals are generated from the abstraction of hydrogen atoms from macromolecules of PVP K-90 by hydrogen atoms and hydroxyl radicals (**Reaction 3**) (Davis et al., 1981; Kadlubowski et al., 2012; Matusiak et al., 2020).



Reaction 1



These polymer radicals are formed at random locations along the chain. When there is no oxygen present in the medium, carbon-centered radicals in aliphatic polymers are involved in biradical reactions such as disproportionation and cross-linking, and also in reactions involving single radicals. Fig. 3 represents the possibilities of formation of a new C–C bond that can occur through intermolecular and intramolecular crosslinking. The first recombination takes place between two radicals of separate macromolecules and the second one is between two radicals in the same polymer chain in different segments of it (Kadlubowski et al., 2012; Matusiak et al., 2020).

The average number of radicals existing in each macromolecule at the same time determines which type of recombination is prevalent in dilute polymer solutions. When the average number is much less than 1 recombination takes place between radicals located in two separate macromolecules (intermolecular crosslinking), since the chance that a radical will find a reaction partner on the same chain is very low. In contrast, intramolecular reactions are favored when at least two radicals are present along the same chain (Kadlubowski et al., 2012). In case of gamma irradiation calculation of the number of radicals present simultaneously on each macromolecule needs some estimations, however approximation can be given. As an example 1 mM PVP K-90 have been considered. Based on polymer concentration, its initial molar mass and absorbed dose (similarly to the procedure presented by Kadlubowski et al. (2012) one can estimate that totally around 25 radicals are generated at each macromolecule during 25 kGy irradiation in argon and more than 50 in N₂O.

In addition to the average number of radicals, the concentration of PVP K-90 also influences the type of crosslinking. Macroscopic gel has been formed for 100 mM PVP K-90 solutions. For 25 mM PVP K-90 solutions an increase in the hydrodynamic radius both in argon saturation and in nitrous oxide saturation have been observed, indicating the favoring of intermolecular cross-linking as a function of increased polymer concentration.

As previously described, the presence of nitrous oxide in the system doubles generation of hydroxyl radicals, which favors the formation of polymeric radicals. Higher average number of radicals in each macromolecule contributes to the generation of intramolecular cross-linking. More significant increase in the hydrodynamic radius was observed in the absence of nitrous oxide, which demonstrates that this gas favors intramolecular cross-linking.

3.2. Static Light Scattering

Fig. 4, Figs. 6 and 8 shows changes in average radius of gyration (R_g), weight-average molecular weight (M_w) and polymer coil density (ρ_{coil}), respectively, as a function of the applied irradiation dose for different concentrations of PVP under nitrous oxide and argon saturation.

Gamma irradiation of 25 mM PVP solution saturated with nitrous oxide at a dose of 1 kGy resulted in an increase of R_g from 44 to 172 nm which may indicate a prevalence of intermolecular crosslinking since, as mentioned earlier, the concentration of PVP (2.78 g/L) approaches the critical concentration (Kadlubowski et al., 2012). The same behavior was observed in an argon atmosphere, however, with a more expressive increase in the value of the radius of gyration reaching numbers above 200 nm, which indicates once again that in an argon atmosphere intermolecular recombination is favored, while in a nitrous oxide atmosphere intramolecular crosslinking is favored due to the higher number of OH radicals.

The analysis of the mean values of R_g of the samples prepared with 1 and 10 mM PVPK-90 solution revealed a pronounced tendency to decrease of the size for samples prepared with 10 mM PVP K-90 solution in both saturation atmospheres. In argon, the radius of gyration value

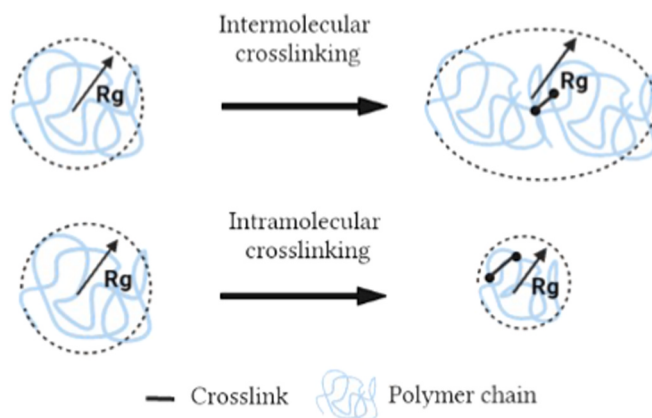


Fig. 5. - Behavior of the radius of gyration as a function of the type of crosslinking.

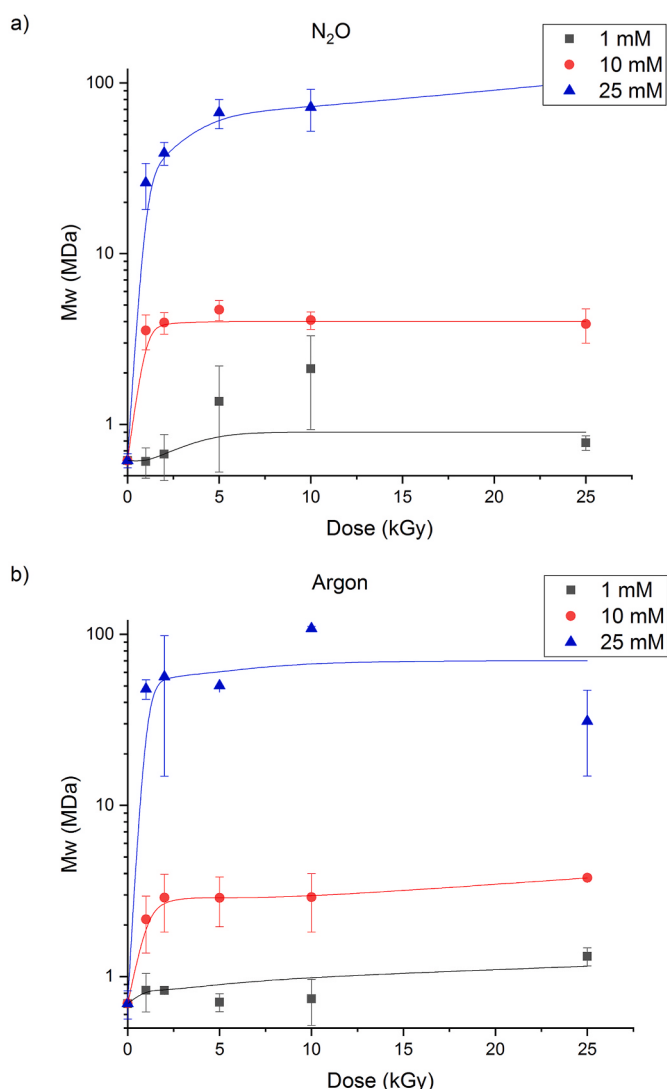


Fig. 6. Changes in weight-average molecular weight (M_w) of PVP as a function of absorbed dose under nitrous oxide and argon saturation. ■ [PVP] = 1 mM; ● [PVP] = 10 mM; ▲ [PVP] = 25 mM. Additional lines were added to guide the eye.

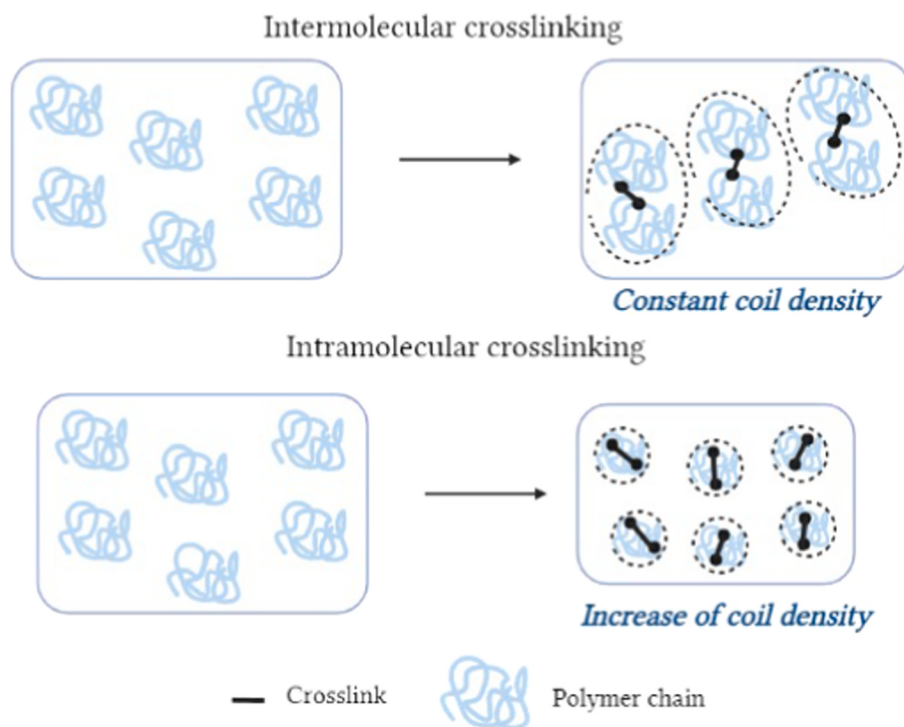


Fig. 7. Behavior of the coil density as a function of the type of crosslinking.

changed from 46 to 44 nm at 1 kGy and then down to 28 nm at 25 kGy. In nitrous oxide, 10 mM PVP K-90 solutions under irradiation of 1 and 25 kGy presented values of 39 and 15 nm, respectively. Reduction of the size, approximately two times in the sample saturated with argon and irradiated with 25 kGy and three times in the sample saturated with nitrous oxide under the same conditions demonstrates once again that the saturation of solutions with N_2O promotes intramolecular crosslinking due to the higher number of hydroxyl radicals in the medium.

There was almost no trend of changes in the radius of gyration of samples prepared with 1 mM PVP concentration as expected since the polymer concentration is low. A possible explanation for this fact is in the possibility of strong competition between the reaction of an OH radical with PVP and with another OH radical. Mutual recombination can occur when OH radicals being formed fail to find PVP as a reaction partner. It is known that in dilute or semi-diluted polymer solutions irradiated with high dose rates in electron beam, the OH radical react with itself with reaction rate constant ($k = 5.5 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$) as described in Reaction 4 (Buxton et al., 1988).



Such a reaction may be prevailing in this condition, since the reaction rate constant of the combination of OH radical with PVP is lower than that of the reaction of OH radical with another OH radical ($k = 2 \times 10^8 \text{ L mol}^{-1} \text{ s}^{-1}$) (Bartoszek et al., 2011).

For the nanogels formation, intramolecular crosslinking of polymer macromolecules is necessary, forming compact, internally crosslinked structures in the form of a 3D-network. This compaction promotes a reduction in the radius of gyration of macromolecules (Fig. 5). The average molecular weight remains constant or it increases when there is some contribution of intermolecular recombination (An et al., 2011; Ashfaq et al., 2021; Matusiak et al., 2020).

The results of radius of gyration reinforced the hypothesis of prevalence of intermolecular crosslinking in samples prepared with a 25 mM PVP K-90 solution, since, in addition to the increase observed in the values of hydrodynamic radius, an increase in the radius of gyration was also observed.

The weight-average molecular weight (M_w) of the samples prepared

with 10 mM PVP K-90 solution remained almost stable for doses higher than 1 kGy evaluated in both argon and nitrous oxide saturation, contributing to the hypothesis of a predominance of intramolecular crosslinking in these solutions. On the other hand, an increase in the weight-average molecular weight (M_w) of the samples prepared with 25 mM PVP K-90 solution was observed, evidencing the prevalence of intermolecular crosslinking in these samples.

Gamma irradiation of 25 mM PVP solution saturated with nitrous oxide at a dose of 5 kGy resulted in an increase of M_w from 0.7 to 67.0 MDa. Similar behavior was observed in an argon atmosphere: increase up to 48.0 MDa at 1 kGy.

Another parameter important for evaluation of the formation of nanogels is the coil density, which can be calculated using Equation (1):

$$q_{\text{coil}} = \frac{M_w}{\frac{4}{3} \pi (R_g)^3} \quad 1$$

where: M_w - weight-average molecular weight and R_g - radius of gyration.

This density corresponds to the density of equivalent sphere of the same mass as the macromolecule and of a radius equal R_g (not including the solvent present in this sphere) (Kadlubowski et al., 2012). Coil density is also understood as polymer mass divided by the volume it occupies. The coil density in nanogels is much higher than the coil density of initial non-crosslinked coils of the parent PVP (Ashfaq et al., 2021; Kadlubowski et al., 2012). Fig. 7 presents the behavior of the coil density as a function of the type of crosslinking obtained.

The coil density of the samples prepared with 1 and 25 mM PVP K-90 solution in both argon and nitrous oxide saturation did not present a significant difference, remaining constant for all applied doses. An increase in the coil density of the samples prepared with a PVP concentration of 1 mM was expected because it was a more dilute solution, but as explained above, it is possible that there is a very strong competition between the reaction of an OH radical with PVP and with another OH radical, the second option being predominant due to the high reaction rate constant.

Gamma irradiation of 10 mM PVP solution saturated with argon at a

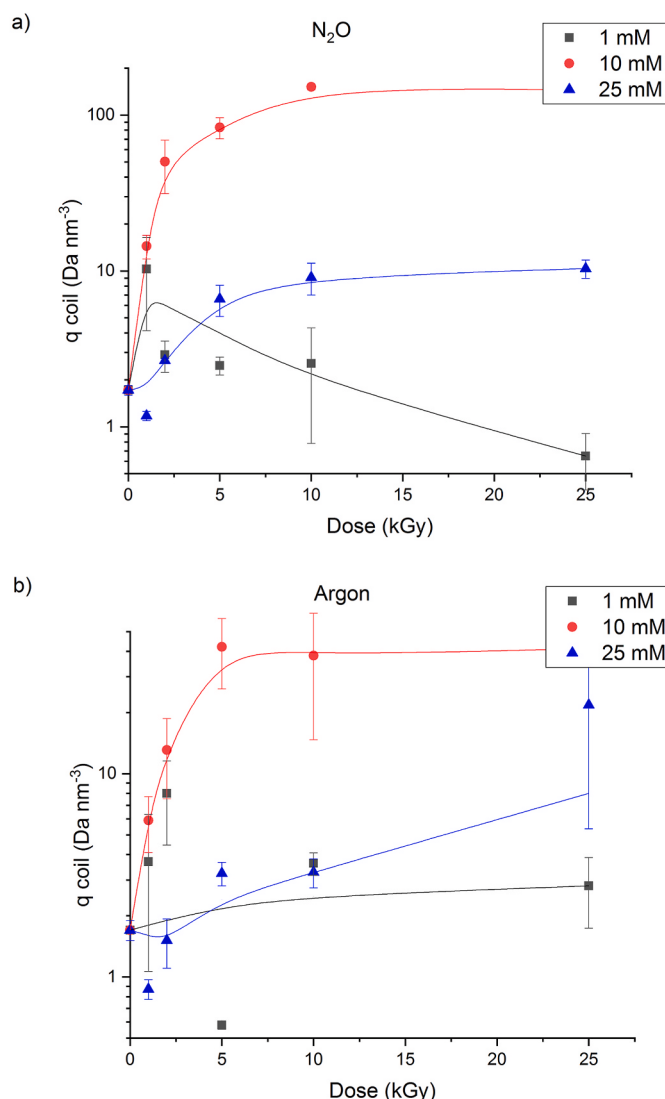


Fig. 8. Changes in polymer coil density (ρ_{coil}) of PVP as a function of absorbed dose under nitrous oxide and argon saturation. ■ [PVP] = 1 mM; ● [PVP] = 10 mM; ▲ [PVP] = 25 mM. Additional lines were added to guide the eye.

dose of 5 kGy resulted in an increase of ρ_{coil} from 1.7 to 42.1 Da nm⁻³. The same behavior was observed in a nitrous oxide atmosphere reaching the value of 151 Da nm⁻³. These data contributed to the verification of intramolecular crosslinking formation in these samples and again it was verified that the saturation in nitrous oxide favors the predominance of intramolecular crosslinking in the system since higher coil density values were obtained with this gas.

The ratio of radius of gyration to the hydrodynamic radius, R_g/R_h , also known as the shape factor, is a dimensionless value that can provide information about the shape and conformation of particles. Determining this ratio (Fig. 9) can then provide insight into the internal structure of nanogels (Burchard, 1999; Kadłubowski et al., 2012; Matusiak et al., 2020; Niezabitowska et al., 2020).

A reduction in the R_g/R_h ratio was observed at all concentrations of PVP K-90. In samples prepared with 1 mM PVP K-90 solution, one can observe decrease from 2.48 (polydisperse polymer sample) to 0.79. This value is characteristic for nano- or microgels (Burchard, 1999).

Samples prepared with 10 mM PVP K-90 solution showed a reduction in the R_g/R_h ratio after irradiation up to 1 which corresponds to homogeneous (hard) spheres, which are much more densely packed than the initial coils of linear macromolecules, characteristic for internally crosslinked chains. Samples prepared with 25 mM PVP K-90 solution

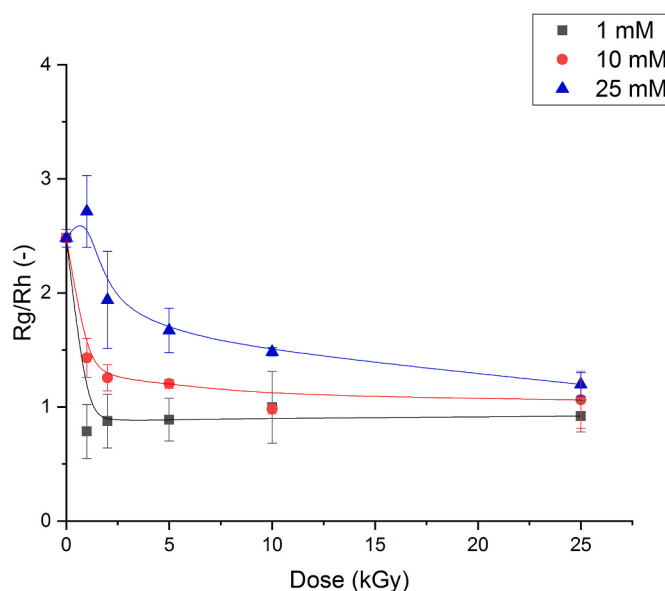


Fig. 9. Changes in R_g/R_h ratio of PVP as a function of absorbed dose under nitrous oxide saturation. ■ [PVP] = 1 mM; ● [PVP] = 10 mM; ▲ [PVP] = 25 mM. Additional lines were added to guide the eye.

showed a reduction in the R_g/R_h ratio up to 1.2 after irradiation with 25 kGy.

The joint analysis of the studied parameters indicated that the 10 mM concentration of PVP K-90 showed the greatest evidence of prevalence of intramolecular crosslinking with constancy in weight-average molecular weight (M_w), tendency to decline in radius of gyration (R_g), increase in polymer coil density (ρ_{coil}) and shape factor (R_g/R_h) around 1.0 indicating homogenous, internally cross-linked spheres. It was additionally found that the formation of nanogel is more predominant in samples saturated with nitrous oxide.

On the other hand, samples prepared with 25 mM PVP K-90 solution showed an increase in weight-average molecular weight (M_w), hydrodynamic radius (R_h) and radius of gyration (R_g) and constant value of polymer coil density (ρ_{coil}), indicating the prevalence of intermolecular crosslinking in these samples. At this concentration, it was also observed that nitrous oxide favors the formation of intramolecular crosslinking, since in its absence the increase of analyzed values was more pronounced.

An interesting phenomenon has been noted for 1 mM PVP K-90. The observed changes in weight-average molecular weight (M_w), hydrodynamic radius (R_h), radius of gyration (R_g) and polymer coil density (ρ_{coil}) do not allow directly to conclude about the ongoing process of intramolecular crosslinking. However calculated R_g/R_h ratio equal 0.8 is characteristic for nano- or microgels. That could mean that number of radicals formed at each macromolecule is high enough to initiate intramolecular recombination. At the same time, in diluted solution, intermolecular recombination is limited because of long distances between macromolecules – that is why there are no significant changes in weight-molecular weight. It doesn't mean that strongly, internally crosslinked macromolecules do not react by mean of intermolecular recombination as slight increase in radius of gyration can be observed. However one have to remember that these are “z-average” values and even a small number of big molecules or aggregates will influence mean R_g value.

3.3. Atomic Force Microscopy (AFM)

As shown, 10 mM PVP K-90 solutions saturated with nitrous oxide showed highest coil density. Thus, the sample prepared with a dose of 10 kGy was selected for morphological analysis. This sample prepared in

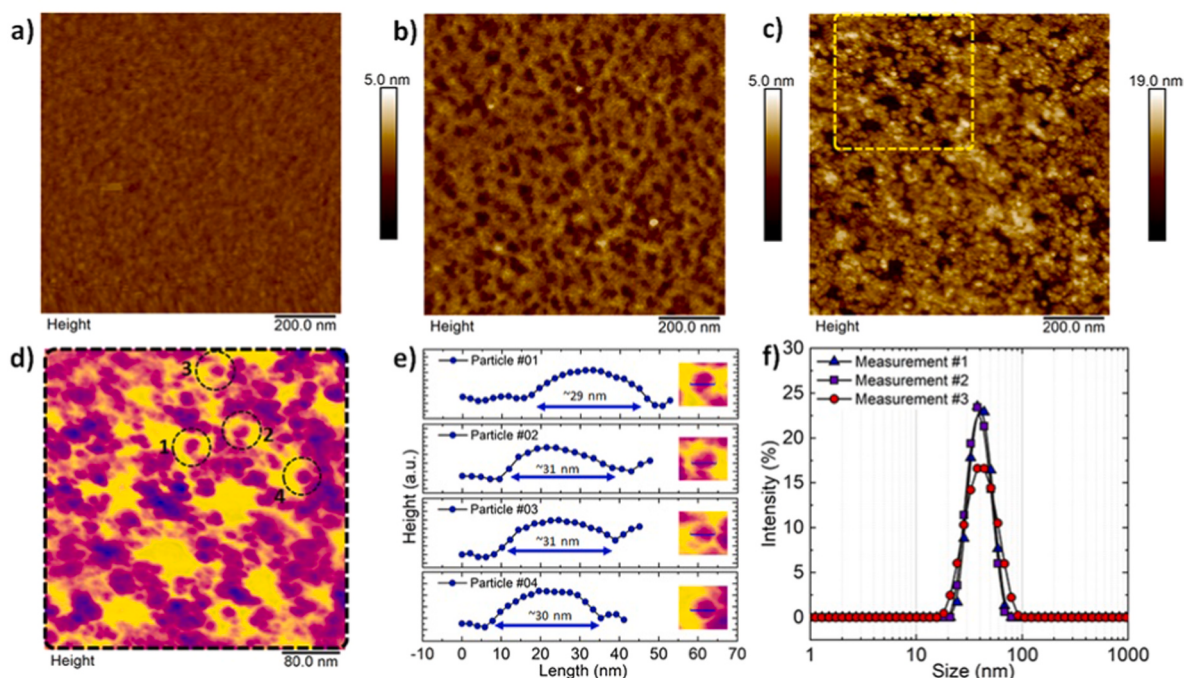


Fig. 10. Morphology analysis by AFM of: (a) the pristine silicon substrate, (b) non-irradiated PVP K-90 thin film @Si, (c) PVP K-90 10 mM nanogel after 10 kGy of gamma irradiation and (d) its zoomed-in (inset) image analysis, (e) particle height profile analysis from previous image and (f) the DLS particle size distribution of the irradiated nanogel solution.

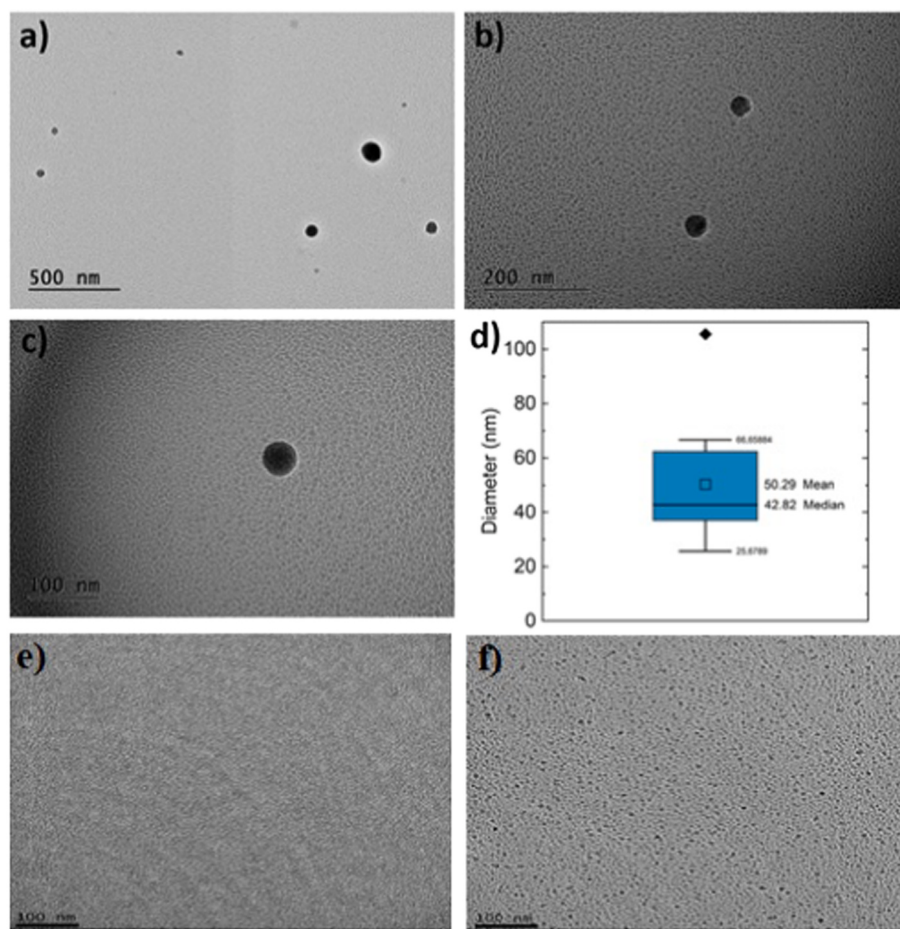


Fig. 11. Morphology by TEM of: (a) (b) (c) PVP K-90 nanogel prepared with 10 mM and 10 kGy of gamma irradiation at different magnification, (d) particle size analysis, (e) carbon-coated copper grid and (f) non-irradiated PVP K-90 solution.

triplicate presented average R_h of 19 nm, average R_g of 19 nm, average ρ_{coil} of 152 and average R_g/R_h ratio of 1. Fig. 10c shows the morphology of this sample compared to the morphology of the non-irradiated PVP K-90 solution (Fig. 10b) and the silicon substrate (Fig. 10a).

The silicon substrate was chosen to obtain the AFM images due to the flat surface without roughness as seen in Fig. 10a. The deposition of PVP K-90 solution on the substrate changed its roughness, forming a kind of low roughness film (Fig. 10b). High relief spherical structures were observed in the images generated with the 10 mM, 10 kGy nanogel sample (Fig. 10c), indicating a completely different behavior in relation to the polymer solution.

Particle size analysis through the AFM image obtained on four particles (Fig. 10d) revealed an average particle size of 30 nm (Fig. 10e) corresponding to the average particle diameter. Thus, the average radius obtained by AFM analysis was 15 nm, a value very close to that obtained by DLS (Fig. 10f) (19 nm), reinforcing the thesis of formation of intramolecular crosslinking in the sample with consequent obtaining of nanogels.

3.4. Transmission Electron Microscopy (TEM)

Fig. 11 shows the morphology by Transmission Electron Microscopy of the sample prepared with 10 mM PVP K-90 solution saturated with nitrous oxide and irradiated with 10 kGy, as well as the morphology of the non-irradiated PVP K-90 solution and the carbon-coated copper grid. It is possible to observe spherical particles with high contrast indicating the formation of nanogel in the sample prepared with 10 mM PVP K-90 solution as seen in the AFM images.

Particle size analysis through the TEM images obtained performed on the particles observed in Fig. 11a, b and 11c revealed an average particle size of 43 nm (median) (Fig. 11d) corresponding to the average particle diameter. Thus, the average radius obtained by TEM analysis was approximately 21 nm, a value that is similar to that obtained in the AFM image and to that determined by DLS. This indicates that nanogels obtained from 10 mM PVP K-90 solution irradiated with 10 kGy are hard, homogenous, non-permeable spheres of very high density as verified by calculation of coil density and R_g/R_h ratio.

4. Conclusion

Poly(N-vinyl pyrrolidone) PVP K-90 nanogels synthesized by radiation-induced intramolecular crosslinking using gamma irradiation were successfully obtained. Samples prepared with 100 mM PVP K-90 solution formed macroscopic gels, in the samples produced with 25 mM PVP K-90 solution there was a prevalence of intermolecular crosslinking. On the other hand, in the samples obtained from 10 mM PVP K-90 solution, there was a predominance of intramolecular crosslinking demonstrated in the tendency to decline in the radius of gyration (R_g), in the constancy of the weight-average molecular weight (M_w), in the increase in polymer coil density (ρ_{coil}), in the R_g/R_h ratio (shape factor) around 1.0 indicating homogenous, internally cross-linked spheres, in the high relief spherical structures observed in the AFM images and in the spherical particles with high contrast observed in the TEM images.

The saturation of the samples with nitrous oxide promoted higher number of hydroxyl radicals, favoring the formation of polymeric radicals. Higher average number of radicals in each macromolecule contributed to the higher yield of intramolecular crosslinking. Thus, it was found that nitrous oxide promotes formation of nanogels.

Author statement

Tatiana Santana Balogh: Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization. **Everton Bonturim:** Methodology, Investigation, Data Curation, Visualization. **Lucas Dias Vieira:** Methodology, Investigation, Data Curation. **Ademar Benévolo Lugão:** Conceptualization, Validation, Resources,

Supervision, Project administration. **Slawomir Kadlubowski:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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