Nano Selenium as antioxidant agent in a multilayer food packaging material

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Abstract

Selenium nanoparticles (SeNPs) were incorporated in a flexible multilayer plastic material using a water-base adhesive as vehicle for SeNPs. The antioxidant performance of both the original solutions containing spherical SeNPs of 50-60 nm diameter, the adhesive containing these SeNPs and the final multilayer plastic material to be used as food packaging was quantitatively measured. The radical scavenging capacity due to SeNPs was quantified by a free radical assay developed in the laboratory and by the diphenyl-1- picrylhydrazyl (DPPH) method. DPPH was not efficient to measure the scavenging capacity in the multilayer when the free radical scavenger is not in the surface in contact with it. Several multilayer laminated structures composed by [PET (20µm) – adhesive – LDPE (with variable thickness from 35 to 90 µm)] were prepared and measured, demonstrating for the first time that free radicals derived from oxygen (OH*, O_2* and O_2H) cross the PE layer and arrive at the adhesive. SeNPs remain as such after manufacture and the final laminate is stable after three months of storage. The antioxidant multilayer is a non-migrating efficient free radical scavenger, able to protect the packaged product versus oxidation and extending the shelf life without being in direct contact with

the product. Migration tests of both Se and SeNPs to simulants and hazelnuts demonstrated the non-migrating performance of this new active packaging.

Keywords: antioxidant, free radical scavenger, selenium nanoparticles, multilayer, DPPH, migration.

1. Introduction

The profound changes in production, distribution and storage of foods as well as the increased society demand that requires good quality products and longer shelf life, leads to the need of new ideas for the packaging sector. Some of these needs have been satisfied with the use of active packaging, either antioxidant or antimicrobial packaging materials. They are classified in two big groups; scavenging systems or releasing systems. The first one is able to remove oxygen, water, ethylene, free radicals, etc. from the package, thus reducing the oxidation processes or early maturation of the packaged food. The other option releases mainly antimicrobial agents, which are capable to inhibit the microbial growth extending its shelf life. In both cases the system consists of incorporating in the packaging material specific substances, usually called active agents, which will act either as antioxidants or antimicrobials for food [1-8]. Although the idea of having the preservatives in the packaging materials instead of in the food is very attractive, to get these materials is very difficult. There are a series of steps to consider and among them the demonstration of the efficiency is the key point. This faces a new analytical challenge, in which quantitative measurements at high level of sensitivity are required for direct application to a packaging material without previous extraction or dissolution. In this work the efficiency of a new antioxidant material containing Se nanoparticles has been demonstrated.

Selenium nanoparticles (SeNPs) can be considered as synthetic antioxidants. Several studies mention their antioxidant capacity [9-12]. SeNPs are able to trap free radicals, increasing their antioxidant effect with decreasing particle size [13,14]. However, all applications and previous measurements were done in direct contact between the SeNPs and the oxidizable matter. Once incorporated into the polymer the homogeneous distribution and size of these SeNPs play a critical role in the antioxidant performance.

This means that the evaluation should be applied first to the initial solution used for producing the material, in order to select the best composition, and later to the material.

The quantitative measurement of antioxidant properties from a solution involves the application of specific analytical methods, such as oxygen radical absorbance capacity (ORAC) [15,16], or total radical trapping antioxidant parameter trapping (TRAP), or electron transfer as TEAC (trolox equivalent antioxidant capacity) [17-19]. However, none of them can be applied to a packaging material containing nanoparticles, as these methods require the extraction of the antioxidant components to a solution in which the reaction takes place. DPPH was developed by Blois [20]. The assay consists of measuring the scavenging capacity of antioxidants towards a stable free radical (α, α-diphenyl-β-picrylhydrazyl). The antioxidants donate a hydrogen atom and thus, the odd electron of nitrogen atom in DPPH is reduced to the corresponding hydrazine [21]. This specie is considered as a stable free radical because it delocalizes the spare electron over the molecule as a whole and does not dimerize, as happens with the majority of free radicals. This method has been used in plastic materials [22], but it was never applied to multilayers, paper or board where there is not direct contact with the antioxidant agent. In 2008 a new analytical method was developed to measure the antioxidant performance directly in the packaging material without extracting the sample.[23,24]. The method consists of exposing the material to an atmosphere enriched in free radicals. The stream of free radicals passes throughout the material to be evaluated and is carried to a salicylic acid solution, where a chemical reaction takes place. The resulting compounds from this chemical reaction are later analyzed by HPLC with fluorescence detector. This way, a quantitative value of the scavenging capacity is achieved. This method was later automatized and now it allows to simultaneously analyze eight samples, where at least one of them has to be a blank without active compounds. The method measures the radical scavenging properties of the material [25-27] and has been applied before to evaluate the performance of several packaging materials in which the antioxidants were incorporated in the external layer of the packaging. A recent study carried out on a multilayer containing green tea extract in an internal layer of the packaging [28,29] demonstrated that the method is also efficient in this case. But the case of nanoparticles is a particular one, what adds a new challenge to be faced.

For this purpose, DPPH and the free radicals method from Pezo were selected to evaluate the antioxidant capacity of multilayers where SeNPs were incorporated. It will be

demonstrated for the first time the advantages and limitations of both methods, which will allow to quantitatively measure the radical scavenging capacity of the new antioxidant multilayer containing SeNPs. In addition, the paper demonstrates the mode of action of the non-migrating antioxidant material as free radicals scavenger and opens new doors to further developments in the use of NPs.

2. Materials and methods.

2.1. Reagents

Sodium salicylate (CAS 54-21-2), 2,5-dihydroxybenzoic acid (2,5-DHB) (CAS 490-79-9), hydrogen peroxide solution (34.01 mg/L), acetic acid (CAS 64-19-7), 2,2-diphenyl-1-picryl-hydrazyl (CAS 1898-66-4) sodium acetate trihydrate (CAS 6131-90-4) and Trolox (CAS 53188-07-1) were purchased from Sigma-Aldrich Química S.A (Madrid, Spain), all of them had analytical quality.

Methanol (high performance liquid chromatography, HPLC grade) was purchased from Scharlau Chemie S.A (Sentmenat, Spain). Purified water was obtained from a Milli-Q 185 Plus system from Millipore (Bedford, MA, USA).

2.2. Solutions of Se NPs and samples

SeNPs were synthesized using a solution-phase approach based on the reduction of selenite with ascorbic acid in the presence of different stabilizers agents polysaccharide (chitosan, a Poly(D-glucosamine)) or an ethoxylated non-ionic surfactant (Triton X-100 (t-octylphenoxypolyethoxy-ethanol)), isotridecanol ethoxylate and/or 2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate. Characterization of the obtained SeNPs by Transmission Electron microscopy showed that the type of agent used for stabilizing SeNPs has a great influence on the size and morphology of the resulting NPs. SeNPs obtained using Chitosan, Triton X100, and 2,4,7,9-tetramethyl 5decyne-4,7-diol ethoxylate were spherical with a diameter size within the range of (20-40), (≈100), (18-40) and (28-60) nm, respectively. The use of isotridecanol ethoxylate as stabilizer agent produced NPs with nanorods morphology (data non-shown), which demonstrated poor antioxidant properties.

Several solutions containing SeNPs were prepared with a composition detailed in table 1. In order to select the most antioxidant formula, the antioxidant capacity of SeNPs solutions was determined by free radical assay and compared with the same solutions but without NPs (blank solutions).

The SeNPs solutions were incorporated into a water-based polyurethane adhesive to manufacture laminates [substrate1-adhesive-substrate2] at laboratory scale. First, different concentrations of SeNPs up to 10% solution of SeNPs were incorporated into the adhesive. Then, it was extended on a 20 x 30 cm substrate (PET of 12 μm thickness) forming a uniform layer using an extender machine (K control coater, RK printcoat instruments). The gramage of adhesive applied was 3 g/m^2 on the laminate, which was gravimetrically controlled. Afterwards, a second 20 x 30 cm substrate of polyethylene of 35, 60 or 90 μm thickness, was placed on top of it and the laminate was pressed at 80ºC and speed number 4 in a BiO 330 Laminator (made in Korea) forming the laminates shown in table 2.

Finally, two laminates with the same composition were thermosealed with a SUPER CELLO (Audion Elektro, Holland) forming a bag of dimensions 1.5dm x1.5dm with inlet and outlet located at 90º to avoid preferential flow ways as well as maximizing turbulence inside the bags, through which the carrier gas enriched with free radicals flow passed. These bags were used to study the influence of the material thickness on the antioxidant capacity, to confirm the mode of action and also for the aging study.

For the study of the antioxidant capacity with DPPH, bags of dimensions 1 x 0.5 dm^2 were prepared in the same manner.

With the aim to discern between selenium endogenous and exogenous from hazelnuts in the migration studies, the synthesis of ^{77}SeNPs was carried out following the same procedure as that applied to the other SeNPs. ^{77}SeNPs solution was incorporated into the adhesive and the built multilayer was used to form the bags filled with hazelnuts and exposed during 10d at 60ºC, according to EU legislation 10/2011 [30].

2.3. Methods

2.3.1. Free radicals assay

In this method (Pezo et al 2006, 2008) [23,24], an atmosphere enriched in free radicals (OH*, O_2* and O_2H) carried by an inert gas passes through the active materials and bubbles into a salicylic acid solution. A home-made device was used for this purpose according to the design described in references 7 & 8. Here, a reaction between the not scavenged free radicals and this salicylic acid solution takes place, producing 2,3-dihydroxybenzoic (2,3-DHB) and 2,5-dihydroxybenzoic (2,5-DHB) acids, among other compounds. The major resulting compound, 2,5-DHB, analyzed by high-performance liquid chromatography (HPLC) coupled to a fluorescence detector, is correlated with the scavenging properties in such a way that the absence of 2,5-DHB in the solution means that radicals were scavenged by the material.

The 2,5-DHB fluorescence signal obtained for each sample was then compared to the reference blank signal. A fluorescent intensity decrease entailed a higher antioxidant capacity of the studied material, because it meant that the material scavenged the free radicals and, therefore, 2,5-DHB was not produced (or it was in a lower extent) in the solution. Then, the antioxidant capacity was indirectly quantitatively evaluated by high-performance liquid chromatography (HPLC) coupled to fluorescence detector where the 2,5-DHB produced, as the major reaction product, was determined in the different solutions. The equipment used was a Waters Separation module (Waters, Milford, MA, USA) coupled to a 474 fluorescence detector (Waters, Milford, MA, USA). The chromatographic separation was carried out with a reversed column phase C18 (100 mm long, 46 mm i.d and 3μm particle size) from Waters. The isocratic mobile phase was a mixture of methanol with aqueous acetate buffer (3.5 mmol/L, pH 5.8) with a 10:90 (v:v) at a flow rate of 1 ml/min. The volume injected was 20 μL. Wavelengths of 324 and 448 μm were used for emission and excitation measurements respectively.

2.3.2. DPPH method

DPPH method was used for determining the free radical-scavenging activity using a stable free radical (α, α-diphenyl-β-picrylhydrazyl). This compound in methanol solution is deep violet colour and becames colourless when mixed with a hydrogen atom donator substance, as an antioxidant. This process can be followed by measuring a decrease in absorbance.

For this purpose a 100 mg/L solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) in methanol was prepared and then 10mL of this was added into a 20 mL glass vials which contained either 15 µl of the solutions with SeNPs (table 1) or 0.1 g of the adhesive containing 10% of the solution with SeNPs (table 1). A DPPH solution without SeNPs was used as control.

All samples were made in triplicate. Before measuring the absorbance, the vials were kept in the dark to avoid light interaction during 2 or 4 hours of reaction time depending on the antioxidant performance. After that, absorbance at 515 nm was measured by an UV-Vis spectrometer (UV-1700 PharmaSpec, Shimadzu) against a blank solution.

The percentage of DPPH inhibition was calculated using the following equation:

$$(DPPH\_concentration_{control} - DPPH\_concentration_{sample})*100/DPPH\ concentration_{control}.$$

2.3.3. Transmission Electronic Microscopy (TEM) and Field emission scanning electron microscopy (FESEM).

The size and morphology of the SeNPs incorporated in the water-based polyurethane adhesive were examined by a high resolution Transmission Electronic Microscopy (TEM) (JEOL-2000 FXII) equipped with an X-Ray Energy Dispersive INCA 200 X-Sight of Oxford Instruments with a resolution from 136 eV to 5.9 KeV.

The SeNPs incorporated in the laminate at laboratory scale were checked by Field emission scanning electron microscopy (FESEM) (Carl Zeiss MERLIN™), equipped with a EDS Detector of X-rays scattered INCA 350 of Oxford Instruments with resolution from 127 eV to 5.9 KeV. The sample was prepared coating the PET substrate with the adhesive containing SeNPs and drying it. The second layer of LDPE was not applied to facilitate the FESEM analysis.

2.4. Determination of antioxidant capacity for the SeNPs solutions and adhesives.

To study the antioxidant capacity of the SeNPs solutions by free radical assay, the different solutions (table 1) were trapped in eight cartridges made from glass tubes (100mm long, 8mm o.d.; 6mm i.d. inlet; 3mm i.d. outlet) containing silanized glass wool on which the SeNPs solutions were spiked. The cartridges were prepared as follows: glass wool (0.1 g) was inserted into the glass tube to get a bed of 2 cm length, then the desired amount of each SeNPs solution (0.4 grams) was added using a long tip Pasteur pipette to

get a complete bed impregnation. The cartridges were installed in the device and the free radicals atmosphere generated passed through them, following the procedure above described.

Eight samples were simultaneously analyzed for each assay. Two of them contained only glass-wool as reference samples, three were filled with solutions without SeNPs (blank) and other three replicates with solutions containing SeNPs. The assay was carried out in the conditions above described (section 2.3.1).

After 48 hours, 2,5-DHB was determined by HPLC-fluorescence and the antioxidant capacity was expressed as hydroxylation percentage. This was calculated by the following formula: (2,5-DHB $area_{sample}$/2,5-DHB $area_{reference}$)*100. Therefore, a lower percentage of hydroxylation entailed a higher antioxidant capacity.

In addition, to verify the antioxidant capacity of SeNPs solutions with a known antioxidant, this assay was repeated under the same conditions above described, but in this case, with Trolox solutions in methanol at increasing concentrations (100µg/g-1000µg/g) to built a calibration plot of the system using Trolox as standard.

DPPH was used to study the antioxidant capacity both SeNPs solutions (table 1) and the adhesives with 10% of these solutions.

For these purpose 15 µL of all solutions (table 1) were added into a 20mL vial with DPPH solution and were analyzed according to the conditions described above (section 2.3.2). To complete the study different concentrations of these solutions were prepared (25-1000 µg/g) and analyzed under the same conditions but in this case centrifugation 4000 rpm for 45 min) and ultrafiltration (Millipore centrifugal filter units, Amicon® Ultra-15. Ultracell 10K, 3500 rpm, 30min) were applied.

To study the influence of SeNPs in the adhesive by DPPH, 0.1 grams of adhesive containing 10 % of these solutions (table 1) and their respective blanks were placed in a 20 ml glass vials and dried on a hot plate for further evaluation. After cooling down, 10 ml of DPPH solution were added to each vial and analyzed according to conditions described above (section 2.3.2) together with centrifugation and ultrafiltration tests.

2.5. Determination of antioxidant capacity of the laminates.

The type of SeNPs selected and its respective blank were incorporated into the adhesive to manufacture the multilayer plastic bags above mentioned. For each assay, eight bags with the same thickness of LDPE, corresponding to 35 μm, were prepared and analyzed simultaneously. Two out of them were prepared with blank adhesive, lam 0 (reference), three bags were built with adhesive containing 10% of solution without SeNPs (lam 1) and three with adhesive containing 10% of solution of SeNPs (lam 2). This assay was repeated for the different thicknesses of LDPE (60 and 90 μm) in order to study the influence of LDPE thickness on the radical scavenging capacity and to demonstrate that free radicals can diffuse through the LDPE layer. Table 2 describes the composition of each laminate used in the study.

Then, several bags of 1 x 0.5 dm^2, containing SeNPs and the control, were filled with 15 ml of the DPPH solution. The bags were placed in 125 mL amber vials to avoid light effect. The percentage of DPPH inhibition was calculated as in section 2.4.

2.6. Aging study of SeNPs in the adhesive.

An aging test to evaluate the efficiency of the SeNPs over time was performed. Several mixtures of the adhesive with 10% of the different SeNPs solution and their respective blanks were stored in vials at room temperature, 23ºC, and without exposure to light. Approximately every fifteen days, the blank plastic bags and the those SeNPs laminates with 60 μm LDPE (lam 0, lam 3 and lam 4) were evaluated again by the free radical assay. Then, the antioxidant capacity was evaluated and monitored over time.

2.7. Specific migration analysis

Specific migration analyses were carried out with two food simulants as well as with a real food, hazelnuts. Different laminates were prepared as [PET (12μm) - Adhesive (with and without Se nanoparticles) – LDPE (35, 60 and 90 μm)]. The laminates which contained adhesive without SeNPs were considered as a procedural blank. Bags were built from these laminates, with dimensions 1 dm x 0.5 dm and were filled with 30 ml of food simulant (either EtOH 50% or HAc 3%), or with 10 grams of grinded hazelnuts. Three independent replicates of each sample were analyzed.

The assays were made according to the European Regulation 10/2011 EU [30]. The filled bags were introduced in an oven and maintained at 60ºC for 10 days. In the case of migration to food simulants an aliquot of each sample was transferred to a glass vial, evaporated in a hot plate at 90ºC and redissolved in HNO_3 1%. The Se concentration in this last solution was measured by ICP-MS. The selected mass of Se was 78. A Se calibration curve in HNO_3 1% (from 0 to 104.77 ng/g) was prepared from a Fluka selenium standard with a concentration of 988 µg/g. The limit of detection (LOD) calculated as three times the standard deviation of the blank by the slope of the calibration curve was 2.32 $ng.g^{-1}$.

The natural concentration of selenium in nuts and hazelnuts is usually high [31,32]. With the aim to evaluate the possible migration of SeNPs from the multilayer packaging (PET-adhesive+SeNPs-LDPE), ^{77}SeNPs were synthesized in order to discern exogenous selenium from the endogenous one already present in the sample. Endogenous content of selenium in hazelnuts was 120 µg·Kg-1 on hazelnuts under study. ^{77}SeNPs were synthesized and laminates were produced in the same manner as for the rest of migration studies. The possible migration of ^{77}SeNPs incorporated into the packaging from bags to hazelnuts was also evaluated by ICP-MS. After the storage in the packaging with different LDPE thicknesses under the conditions previously mentioned, an aliquot of 500 mg of hazelnut sample was digested with concentrated HNO_3 (Merck) and 30% hydrogen peroxide (Panreac) (5:1 v:v) in double walled advanced composite vessels (ACV), using a 1000 W microwave sample preparation (MS) microwave oven (CEM, Mattheus, NC). The resulting solution was diluted with deionized water and ^{77}Se was determined by ICP-MS. Moreover, samples on packages without ^{77}SeNPs were also used as control. The results are expressed as the mean value (standard deviation for n=3) (LOD ≈ 0.46 ng Se·ml-1 and LOQ ≈ 1.53 ng Se·ml-1)

3. Results and Discussion

The principle of antioxidant behavior in this work is based on free radicals scavenging, following the theory described by C. Nerín [33]. SeNPs incorporated in the adhesive are non-migrating free radical scavengers and consequently they act as good antioxidants, as will be demonstrated. First, the antioxidant capacity for SeNPs solutions and adhesives containing SeNPs with different stabilizing agents was tested by both the DPPH and free

radical assays. The solution providing the maximum antioxidant capacity was incorporated into the adhesive. This active adhesive was later used to build the multilayer (laminates) of different thicknesses. This way SeNPs were incorporated in the adhesive applied on the internal layer of the laminate. Consequently, the scavenging properties imply that the free radical cross the LDPE layer and arrive at the adhesive layer,where they can be scavenged by the SeNPs. To demonstrate this mode of action several laminates with increasing thicknesses of LDPE layer were tested. Fig. 1 confirms that the radical scavenging capacity decreases when the thickness increases. Diffusion through the LDPE layer of oxo, hydroxo and peroxo radicals is efficiently achieved and then these free radicals arrive at the adhesive behind the LDPE layer and are scavenged. This behavior is not surprising. Diffusion coefficients of many organic compounds throughout LDPE layer have been measured in several papers [34-36] and it has been demonstrated that diffusion of organic molecules in LDPE are usually very fast. Of course this diffusion depends on the molecular mass and volume and also on the polarity of the specific molecule. But the main variable affecting is the size of the molecule. The case of small radicals such as OH*, O_2* and O_2H* is even faster, although the corresponding diffusion coefficients were never measured. The experimental data showed that in fact these radicals cross the LDPE and arrive at the adhesive layer where the nanoSe is anchored. When increasing the thickness of LDPE the diffusion takes more time and some of the free radicals are probably lost, what results in decreasing the scavenging capacity. However, when the free radicals are big, such as that of DPPH*, they cannot cross the LDPE layer, as demonstrated in this study. Although the antioxidant performance of SeNPs has been demonstrated by other authors [9-12] this is the first time that the behavior of nanoparticles without direct contact with the free radicals has been demonstrated. The stability of the SeNPs over time was examined as well. Both studies were carried out by free radical assay and DPPH method and the results are shown below.

3.1. Determination of antioxidant capacity for the SeNPs solutions and adhesives.

3.1.1. Free radical assay

Four solutions (1 to 4) containing 100 $mg.L^{-1}$ SeNPs synthesized in presence of different stabilizing agents were used to evaluate the scavenging capacity by the DPPH and free radical assay and the results were compared to those of the respective control.

The results obtained by the free radical assay are shown in Figure 1, where the antioxidant capacity is expressed as % hydroxylation. Clean glass-wool without any solution was used as blank reference (100% hydroxylation when there is not antioxidant capacity), and the rest were the solutions with or without SeNPs. A decrease of hydroxylation % is correlated to an increase of antioxidant capacity [24].

Those solutions without SeNPs demonstrated to have certain antioxidant capacity, which can be attributed to the capacity as scavengers of free radicals of the reagents used in the synthesis of SeNPs used as well as control samples..

Only solution 4, among the four solutions containing 100 $mg.L^{-1}$ SeNPs, showed significant differences compared to the respective blank. Its hydroxylation percentage was lower than the control (53 % vs 70 %), so its antioxidant capacity was higher. This result demonstrates that the 2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate, reagent used to stabilize the SeNPs solution, was the most appropriate stabilizer. This percentage of hydroxylation (53%) corresponds to 500µg/g of Trolox in methanol.

Taking into account this positive result, higher concentrations of SeNPs, 750 and 1000 $mg.L^{-1}$(solutions 5 and 6), were tested (Figure 1). The fact that an increase of SeNPs did not provide, as expected, an enhance of its antioxidant capacity was attributed to the limited concentration of 2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate, as it was the same as that used for 100 $mg.L^{-1}$ Se NPs, which was not enough for a complete SeNPs stabilization.

Thus, the percentage of 2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate in the solution of 1000 $mg.L^{-1}$ was increased to 1% (solution 7 and 8) and 7 % (solution 9 and 10) at two different pHs. The results obtained, also shown in Figure 1, evidenced that the solutions with a higher percentage (7%) of stabilizing agent (solutions 9 and 10) provided very encouraging results, as the hydroxylation percentage decreased to 14 and 19% for 1 and 7% respectively. These results demonstrated that SeNPs act as very good antioxidant agents when nanoparticles are well estabilized.

The non-migrating concept applied to SeNPs in this paper has been demonstrated by specific migration analysis with both food simulants and real food (hazelnuts), using ^{77}Se to produce the SeNPs in the case of migration to hazelnuts and ICP-MS as analytical technique. The details of this study are shown below.

3.1.2. DPPH Method

The antioxidant capacity of all the above mentioned solutions and adhesive containing SeNPs was tested (alone and once incorporated in the adhesive) by the DPPH method. However, the results obtained were not coherent with those previously obtained by the free radical assay. For both the solutions and the adhesives, DPPH inhibition of the blank was higher than that of the solution or the adhesive with SeNPs. Also, as SeNPs concentration increases, the percentage of DPPH inhibition increases, and so, the antioxidant capacity. The results obtained for solutions with different concentrations of SeNPs prepared from solution 9 and its respective blank are shown in Figure 2a. In addition to the mentioned lower antioxidant capacity for higher concentrated solutions, the standard deviation of the three replicates was high. Similar results were obtained for solutions with different concentrations prepared from solutions 7, 8 and 10.

One possible explanation is that the nanoparticles interfere with the measurement in the spectrophotometer producing light scattering effect. Consequently, the absorbance at 515 nm will be higher than that expected. If this happens, the measured concentration of DPPH will be higher than the real one, thus creating an optical interference.

Two strategies were applied to avoid this problem: centrifugation (4000 rpm for 45 min) and ultrafiltration of the solutions (Millipore centrifugal filter units, Amicon® Ultra-15. Ultracell 10K, 3500 rpm, 30 min) in both cases after the reaction time.

The results obtained by centrifugation were not successful but after ultrafiltration and removal of the SeNPs from the solution, the results obtained were coherent with those obtained by the free radical assay (Figure 2b), and the antioxidant capacity of the adhesives increased with increasing SeNPs concentration, as expected. Also, the lower standard deviation between the three replicates confirmed the previous hypothesis. These results indicate that the DPPH method is not suitable for the analysis of antioxidant capacity of solutions containing nanomaterials in a single step, while the presence of nanoparticles does not interfere with the free radical assay.

A concentration of 100 $mg.L^{-1}$ of SeNPs in the adhesive was selected as the optimum. In addition, previous studies of compatibility with the adhesive established 10% as the maximum amount of solution that can be added to the adhesive without losing any

adhesion capacity. The antioxidant capacity of the adhesives prepared with the different solutions in which 2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate is used as stabilizing agent, after ultrafiltration, are shown in Figure 2c. In all cases, adhesives prepared with blank solutions presented some antioxidant capacity. No significant differences in antioxidant capacity were found between the solutions prepared with different concentrations of stabilizing agent or at different pHs. The antioxidant capacity was slightly higher for the adhesive prepared with solution 7. However, according to the results obtained by the free radical assay, solution 9 was selected as the optimum one to continue with the rest of the study and manufacture the laminates.

3.2. Antioxidant capacity of the laminates and influence of LDPE thickness.

Once the solution with the highest antioxidant capacity (solution 9) was selected, an antioxidant water-base polyurethane (PU) adhesive with 10% of solution 9 and its respective blank was prepared. Laminates with different LDPE thicknesses (35, 60 and 90µm) were tested, as described in section 2.2, and their antioxidant capacity was evaluated by both the free radical assay and DPPH method.

Figure 3 shows the % hydroxylation (lam 0 as reference=100%) together with the different laminates (see Table 2 for description), with and without SeNPs. As can be seen, the blank laminates (without SeNPs) possess a certain antioxidant capacity because the percentages of hydroxylation of lam 1, 3 and 5 were lower than those of laminate 0 (used as reference). This result is in good agreement with that obtained for the blank solutions without SeNPs, (section 3.1.1), and is attributed to the presence of some antioxidant compounds, different from SeNPs, in the composition.

A TEM photograph of SeNPs in the adhesive was obtained (Figure 4), where spherical nanoparticles can be clearly seen. The X ray spectrum identifies the Se in the nanoparticles as shows Figure 4.

Furthermore, the hydroxylation percentage decreases when the thickness of LDPE layer decreases (lam 2< lam 4 < lam 6). This behavior demonstrates for the first time that the free radicals from oxygen (OH*, O_2* and O_2H), which have a molecular mass between 17 and 33 amu, are able to cross the LDPE layer and diffuse through it, arrive at the adhesive where SeNPs were inserted and be scavenged when thin LDPE layers are used.

The percentage of DPPH inhibition obtained (not shown) was very similar for all the laminates studied and no significant differences with the adhesive with and without SeNPs or with a bag of 60 μm or 35 μm LDPE were observed. The large size of DPPH with a molar mass of 394.32 g/mol, prevent its diffusion through the LDPE layer and so its penetration to reach the layer of adhesive behind the LDPE layer, where the SeNPs were placed. This performance opens new doors to the concept of antioxidant as radical scavenger, in which the molar mass of free radicals can be a limiting factor when no direct contact occurs. However, it can be pointed out that the oxidation process starts from the free radicals derived from oxygen. Other organic free radicals, such as lipid and lipid peroxyl radicals, are formed from the reaction between the lipids and those free radicals derived from oxygen. However, the early free radicals initiating the oxidation reaction are always those derived from oxygen. Lipid and lipid peroxyl radicals can be considered as secondary initiators, probably part of the propagation step in the oxidation reaction. Consequently, the oxidation protection can be achieved just using the scavengers for oxygen derived species. In this case, the lack of direct interaction between SeNPs-DPPH justifies the negative response given by DPPH. Thus, it can be confirmed that the DPPH method is not suitable for measuring the antioxidant capacity in multilayer materials where the radical scavenger is not in direct contact with the reagent. It is important to point out that it is the first time that this limitation of DPPH method is reported.

3.3. Aging study of SeNPs in the adhesive.

Once the antioxidant capacities of the SeNPs applied to the laminates were confirmed, the stability of the adhesives over time was evaluated. This is a critical and important feature for further industrial use, due to the elapsed time from their production to their final end-user application to prepare the new manufactured antioxidant food packaging materials

Samples prepared with the new antioxidant adhesive with SeNPs or blank solutions were stored and analyzed at approximately fifteen days intervals. It was observed that SeNPs sedimented with time at the bottom of the vial being within thirty days, most of them already deposited at the bottom. Therefore, the vial was sonicated for approximately 30 minutes to obtain a homogenous adhesive sample before application. Laminates of 60 μm LDPE thickness were then manufactured and analyzed by free radical assay.

Figure 5 shows the results of antioxidant capacity obtained at the different storage times. As can be observed, the percentages of hydroxylation for lam 4 increased from 40% to 90% over time, which evidences a considerable decrease in antioxidant performance. These results showed a close relationship between the decrease in antioxidant capacity of SeNPs laminates and the sedimentation of nanoparticles. After 62 days, the percentage of hydroxylation found for lam 4 was 90%, similar to that obtained with the blank, so the SeNPs were not anymore in the adhesive and thus the antioxidant capacity was completely lost. This may be due to an agglomeration of the SeNPs with time, not even able to separate into individual nanoparticles after sonication. This result can be very useful for the suppliers of these materials as it is advised not to keep the adhesive containing SeNPs stored longer than one month.

The aging process of the laminate once produced with SeNPs was also studied. As can be seen in Figure 5, the laminates were stable for three months, what emphasizes the importance of this development.

Also, a FESEM photograph of SeNPs in the laminate is shown (Figure 4), where can be seen that SeNPs are distributed in the material retaining their original shape and size.

3.4. Migration studies

The results obtained in the migration tests to food simulants showed that in all cases the migration of ^{78}Se was below the LOD. When migration tests were done in hazelnuts and ^{77}Se was measured, the results on the ratio $^{77}Se/^{78}Se$ on hazelnuts packaged in the new antioxidant material and those packaged in blank material (without SeNPs) were similar and no significant differences were found between controls (samples contained in a packaging without SeNPs) and active packaging with $^{77}SeNPs$ incorporated. Moreover, the material thickness did not influence the SeNPs migration in any case. Thus, migration of SeNPs was not observed from multilayer packaging to food or food simulants after the migration assays at 60ºC for 10 days. This was expected, as the SeNPs were anchored behind the LDPE layer in the multilayer material and the nanoparticles cannot diffuse in the non-polar LDPE layer [37]. If the nanoparticles were incorporated directly in the LDPE layer some low migration could be expected, as has been demonstrated in previous publications working with silver nanoparticles or nanoclay [38-41]. However, only the

nanoparticles from the surface migrate either to the simulants or to the food. The additional advantage of this development, where the nanoparticles are not in direct contact with the food, but behind the LDPE layer, is that they cannot migrate. This way, we can guaranty that the material is safe. The same behavior has been demonstrated working with green tea extract anchored in the adhesive behind the LDPE in a multilayer structure [29].

4. Conclusions

The antioxidant capacity based on the capacity of SeNPs as free radicals scavengers has been demonstrated. SeNPs were stabilized and incorporated for the first time in an adhesive, further used for building a multilayer plastic structure of PET-adhesive-LDPE. The antioxidant performance of this multilayer has been studied by both the DPPH method and the free radical assay previously developed [23,24]. The study concludes that DPPH radical is not able to cross the LDPE layer and consequently the DPPH method is not valid for measuring antioxidant capacity when the antioxidant is not in direct contact with the DPPH radical. This behavior could be attributed to the big size of DPPH radical, that is not able to diffuse throughout any plastic layer. It is the first time that such a limitation of the method is described. In addition, when nanoparticles are involved in a solution, DPPH method also shows some spectral interferences, which require ultrafiltration and/or dialysis to remove the nanoparticles from the solution. This drawback was never described before.

The thicker the laminate the lower the antioxidant capacity of the material because free radicals (OH*, O_2* and O_2H) are able to cross more easily thinner laminates of the LDPE layer and efficiently scavenged when reaching the adhesive containing SeNPs. This behavior is demonstrated in this work.

Concerning the SeNPs it has been demonstrated that among the stabilizing agents tested 7% of 2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate at pH 3 is the most appropriate for this application. An increase of nanoparticles concentration also produced an increase in the antioxidant capacity of both the solutions and the adhesives, and so, 100 $mg.L^{-1}$ was selected as the optimal concentration in the adhesive. Higher concentration caused a reduction of the adhesion properties.

The adhesive containing SeNPs is stable for one month, after which and even using sonication the antioxidant capacity is not recovered, probably due to an irreversible aggregation of nanoparticles.

It is important to mention that no migration of SeNPs was found from the laminates to food simulants or real food inside the package.

Finally it is worth pointing out that SeNPs were placed between PET and LDPE in the laminates here studied. The study demonstrates that free radicals (OH*, O_2* and O_2H) cross the PE layer, but this is unlikely happens with PET, what makes possible the long lasting of the laminates, as the SeNPs remain unchanged in the laminate.

Many food applications could benefit from this new antioxidant material, as its mechanical, optical and machinability performance are exactly the same as those usually required for any application. Then, fresh meat, ready to eat, dried nuts, snaks and everything susceptible of oxidation can be packaged with this new material. Transparency and color are not compromised and the SeNPs are neither visible, nor perceived.

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Bibliography

1. Vermeiren L, Devlieghere F, van Beest M, de Kruijf N, Debevere J (1999) Developments in the active packaging of foods. Trends Food Sci Technol 10 (3):77-86. doi:10.1016/s0924-2244(99)00032-1
2. Ozdemir M, Floros JD (2004) Active food packaging technologies. Crit Rev Food Sci Nutr 44 (3):185-193. doi:10.1080/10408690490441578
3. Lee SY, Lee SJ, Choi DS, Hur SJ (2015) Current topics in active and intelligent food packaging for preservation of fresh foods. J Sci Food Agric 95 (14):2799-2810. doi:10.1002/jsfa.7218
4. Karaman AD, Ozer B, Pascall MA, Alvarez V (2015) Recent Advances in Dairy Packaging. Food Rev Int 31 (4):295-318. doi:10.1080/87559129.2015.1015138

5. Biji KB, Ravishankar CN, Mohan CO, Gopal TKS (2015) Smart packaging systems for food applications: a review. J Food Sci Technol-Mysore 52 (10):6125-6135. doi:10.1007/s13197-015-1766-7
6. Cichello SA (2015) Oxygen absorbers in food preservation: a review. J Food Sci Technol-Mysore 52 (4):1889-1895. doi:10.1007/s13197-014-1265-2
7. Valdes A, Mellinas AC, Ramos M, Burgos N, Jimenez A, Garrigos MC (2015) Use of herbs, spices and their bioactive compounds in active food packaging. RSC Adv 5 (50):40324-40335. doi:10.1039/c4ra17286h
8. Realini CE, Marcos B (2014) Active and intelligent packaging systems for a modern society. Meat Sci 98 (3):404-419. doi:10.1016/j.meatsci.2014.06.031
9. Bai Y, Qin BY, Zhou YH, Wang YD, Wang Z, Zheng WJ (2011) Preparation and Antioxidant Capacity of Element Selenium Nanoparticles Sol-Gel Compounds. J Nanosci Nanotechnol 11 (6):5012-5017. doi:10.1166/jnn.2011.4130
10. Torres SK, Campos VL, Leon CG, Rodriguez-Llamazares SM, Rojas SM, Gonzalez M, Smith C, Mondaca MA (2012) Biosynthesis of selenium nanoparticles by Pantoea agglomerans and their antioxidant activity. J Nanopart Res 14 (11). doi:1236 10.1007/s11051-012-1236-3
11. Sarkar B, Bhattacharjee S, Daware A, Tribedi P, Krishnani KK, Minhas PS (2015) Selenium Nanoparticles for Stress-Resilient Fish and Livestock. Nanoscale Res Lett 10:14. doi:10.1186/s11671-015-1073-2
12. Forootanfar H, Adeli-Sardou M, Nikkhoo M, Mehrabani M, Amir-Heidari B, Shahverdi AR, Shakibaie M (2014) Antioxidant and cytotoxic effect of biologically synthesized selenium nanoparticles in comparison to selenium dioxide. J Trace Elem Med Biol 28 (1):75-79. doi:10.1016/j.jtemb.2013.07.005
13. Huang B, Zhang JS, Hou JW, Chen C (2003) Free radical scavenging efficiency of Nano-Se in vitro. Free Radic Biol Med 35 (7):805-813. doi:10.1016/s0891-5849(03)00428-3
14. Yu B, You PT, Song MF, Zhou Y, Yu FQ, Zheng WJ (2016) A facile and fast synthetic approach to create selenium nanoparticles with diverse shapes and their antioxidation ability. New Journal of Chemistry 40 (2):1118-1123. doi:10.1039/c5nj02519b
15. Bentayeb K, Vera P, Rubio C, Nerin C (2009) Adaptation of the ORAC assay to the common laboratory equipment and subsequent application to antioxidant plastic films. Anal Bioanal Chem 394 (3):903-910. doi:10.1007/s00216-009-2766-0
16. Bentayeb K, Vera P, Rubio C, Nerin C (2014) The additive properties of Oxygen Radical Absorbance Capacity (ORAC) assay: The case of essential oils. Food Chem 148:204-208. doi:10.1016/j.foodchem.2013.10.037
17. Schaich KM, Tian X, Xie J (2015) Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays (Reprinted). J Funct Food 18:782-796. doi:10.1016/j.jff.2015.05.024
18. Cuvelier ME, Maillard MN (2007) How is evaluated the activity of food antioxidants? A review. Sci Aliments 27 (4-5):259-282. doi:10.3166/sda.27.259-282
19. Huang DJ, Ou BX, Prior RL (2005) The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry 53 (6):1841-1856. doi:10.1021/jf030723c
20. Blois MS (1958) ANTIOXIDANT DETERMINATIONS BY THE USE OF A STABLE FREE RADICAL. Nature 181 (4617):1199-1200. doi:10.1038/1811199a0
21. Contrerasguzman ES, Strong FC (1982) DETERMINATION OF TOCOPHEROLS (VITAMIN-E) BY REDUCTION OF CUPRIC ION. Journal of the Association of Official Analytical Chemists 65 (5):1215-1221
22. Lopez de Dicastillo C, Rodriguez F, Guarda A, Galotto MJ (2016) Antioxidant films based on cross-linked methyl cellulose and native Chilean berry for food packaging applications. Carbohydrate polymers 136:1052-1060. doi:10.1016/j.carbpol.2015.10.013
23. Pezo D, Salafranca J, Nerin C (2006) Design of a method for generation of gas-phase hydroxyl radicals, and use of HPLC with fluorescence detection to assess the antioxidant

capacity of natural essential oils. Anal Bioanal Chem 385 (7):1241-1246. doi:10.1007/s00216-006-0395-4
24. Pezo D, Salafranca J, Nerin C (2008) Determination of the antioxidant capacity of active food packagings by in situ gas-phase hydroxyl radical generation and high-performance liquid chromatography-fluorescence detection. J Chromatogr A 1178 (1-2):126-133. doi:10.1016/j.chroma.2007.11.062
25. Gomez-Estaca J, Lopez-de-Dicastillo C, Hernandez-Munoz P, Catala R, Gavara R (2014) Advances in antioxidant active food packaging. Trends Food Sci Technol 35 (1):42-51. doi:10.1016/j.tifs.2013.10.008
26. Komagoe K, Takeuchi H, Katsu T (2008) Oxygen electrode as a new tool to evaluate hydroxyl radical-scavenging ability. Sensors and Actuators B-Chemical 134 (2):516-520. doi:10.1016/j.snb.2008.05.033
27. Liang AH, Zhang NN, Jiang ZL, Liu RJ (2008) Nanosilver resonance scattering spectral method for determination of hydroxyl radical and its application. Science in China Series B-Chemistry 51 (3):226-232. doi:10.1007/s11426-008-0005-8
28. Carrizo D, Gullo G, Bosetti O, Nerín C (2014) Development of an active food packaging system with antioxidant properties based on green tea extract. Food Additives & Contaminants: Part A 31 (3):364-373. doi:10.1080/19440049.2013.869361
29. Carrizo D, Taborda G, Nerin C, Bosetti O (2016) Extension of shelf life of two fatty foods using a new antioxidant multilayer packaging containing green tea extract. Innov Food Sci Emerg Technol 33:534-541. doi:10.1016/j.ifset.2015.10.018
30. 10/2011/EC (2011) Commission regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.
31. Tosic SB, Mitic SS, Velimirovic DS, Stojanovic GS, Pavlovic AN, Pecev-Marinkovic ET (2015) Elemental composition of edible nuts: fast optimization and validation procedure of an ICP-OES method. J Sci Food Agric 95 (11):2271-2278. doi:10.1002/jsfa.6946
32. Yin LL, Tian Q, Shao XZ, Kong XY, Ji YQ (2015) Determination of Trace Elements in Edible Nuts in the Beijing Market by ICP-MS. Biomed Environ Sci 28 (6):449-454. doi:10.3967/bes2015.063
33. Nerín C (2010) 16 - Antioxidant active food packaging and antioxidant edible films A2 - Decker, Eric A. In: Elias RJ, McClements DJ (eds) Oxidation in Foods and Beverages and Antioxidant Applications. Woodhead Publishing, pp 496-515. doi:http://dx.doi.org/10.1533/9780857090331.3.496
34. Canellas E, Aznar M, Nerin C, Mercea P (2010) Partition and diffusion of volatile compounds from acrylic adhesives used for food packaging multilayers manufacturing. J Mater Chem 20 (24):5100-5109. doi:10.1039/c0jm00514b
35. Nerin C, Gaspar J, Vera P, Canellas E, Aznar M, Mercea P (2013) Determination of partition and diffusion coefficients of components of two rubber adhesives in different multilayer materials. Int J Adhes Adhes 40:56-63. doi:10.1016/j.ijadhadh.2012.07.003
36. Dole P, Feigenbaum AE, De la Cruz C, Pastorelli S, Paseiro P, Hankemeier T, Voulzatis Y, Aucejo S, Saillard P, Papaspyrides C (2006) Typical diffusion behaviour in packaging polymers - application to functional barriers. Food Addit Contam Part A-Chem 23 (2):202-211. doi:10.1080/02652030500373661
37. Bott J, Stormer A, Franz R (2014) Migration of nanoparticles from plastic packaging materials containing carbon black into foodstuffs. Food Addit Contam Part A-Chem 31 (10):1769-1782. doi:10.1080/19440049.2014.952786
38. Farhoodi M (2016) Nanocomposite Materials for Food Packaging Applications: Characterization and Safety Evaluation. Food Eng Rev 8 (1):35-51. doi:10.1007/s12393-015-9114-2
39. Echegoyen Y, Rodríguez S, Nerín C (2016) Nanoclay migration from food packaging materials. Food Additives & Contaminants: Part A:1-10. doi:10.1080/19440049.2015.1136844

40. Mackevica A, Olsson ME, Hansen SF (2016) Silver nanoparticle release from commercially available plastic food containers into food simulants. J Nanopart Res 18 (1):11. doi:10.1007/s11051-015-3313-x
41. Hannon JC, Kerry JP, Cruz-Romero M, Azlin-Hasim S, Morris M, Cummins E (2016) Assessment of the migration potential of nanosilver from nanoparticle-coated low-density polyethylene food packaging into food simulants. Food Addit Contam Part A-Chem 33 (1):167-178. doi:10.1080/19440049.2015.1114184

Table 1: Composition of the solutions with SeNPs studied.

Solution number	Se NPs concentration	Stabilizing agent	Concentration of stabilizing agent
1	100 ppm	Chitosan	0.1%
2	100 ppm	Triton X-100	0.1%
3	100 ppm	isotridecanol ethoxylate	0.1%
4	100 ppm	2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate	0.1%
5	750 ppm	2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate	0.1%
6	1000 ppm	2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate	0.1%
7	1000 ppm	2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate	1% (pH 3)
8	1000 ppm	2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate	1% (pH 6.5)
9	1000 ppm	2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate	7% (pH 3)
10	1000 ppm	2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate	7% (pH 6.5)

Table 2: Different laminates studied.

Laminate number	Substrate 1	Solution added to Adhesive	Substrate 2
0	PET (12 μm)	-	PE (different thicknesses)
1	PET (12 μm)	10% solution 9 blank	PE (35 μm)
2	PET (12 μm)	10% solution 9	PE (35 μm)
3	PET (12 μm)	10% solution 9 blank	PE (60 μm)
4	PET (12 μm)	10% solution 9	PE (60 μm)
5	PET (12 μm)	10% solution 9 blank	PE (90 μm)
6	PET (12 μm)	10% solution 9	PE (90 μm)

Table 3: Migration results of ^{78}SeNPs for food stimulants and ^{77}SeNPs for grinded hazelnut measured by ICP-MS

Samples	Concentration of ^{78}Se		Concentration of ^{77}Se
	Simulant EtOH 50%	Simulant HAc 3%	10 grams of hazelnuts
Laminate PET (12 µm) /adhesive with SeNPs/ PE (35µm)	<LOD	<LOD	<LOD
Laminate PET (12 µm) /adhesive with SeNPs/ /PE (60µm)	<LOD	<LOD	<LOD
Laminate PET (12 µm) /adhesive with SeNPs/ /PE (90µm)	<LOD	<LOD	<LOD

LOD for ^{78}Se = 2.32 ng/g.
LOD for ^{77}Se = 0.46 ng/ml.

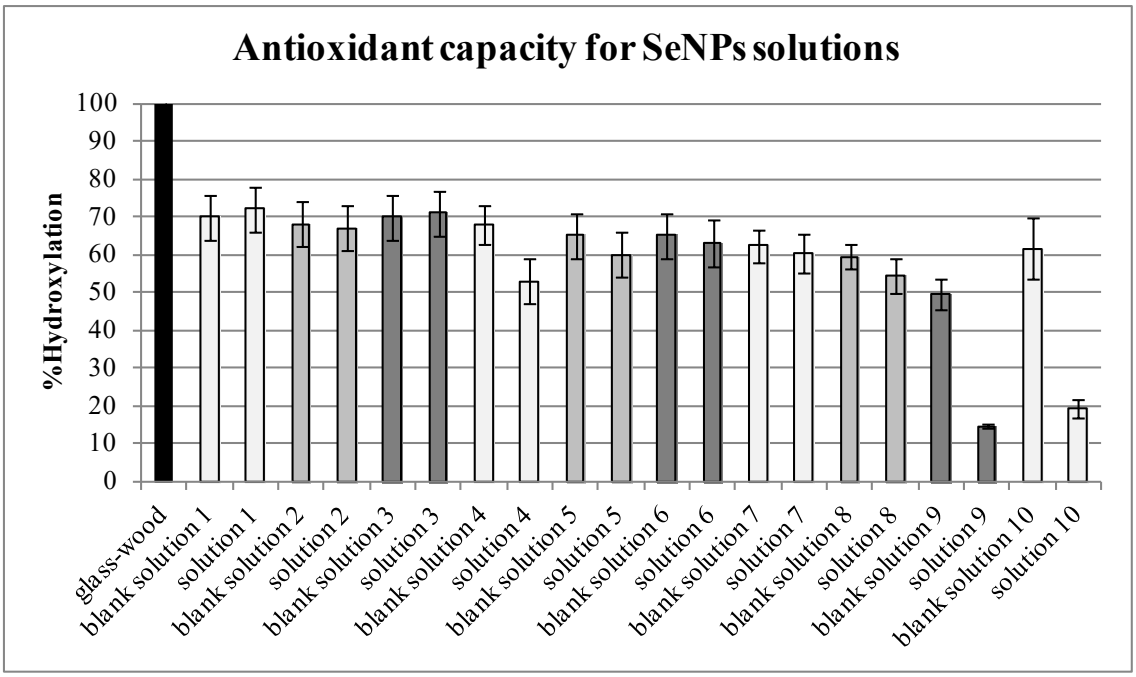


Figure 1: Antioxidant capacity expressed as % hydroxylation for the solution with or without Se NPs synthesized with different stabilizing agents and with different conditions.

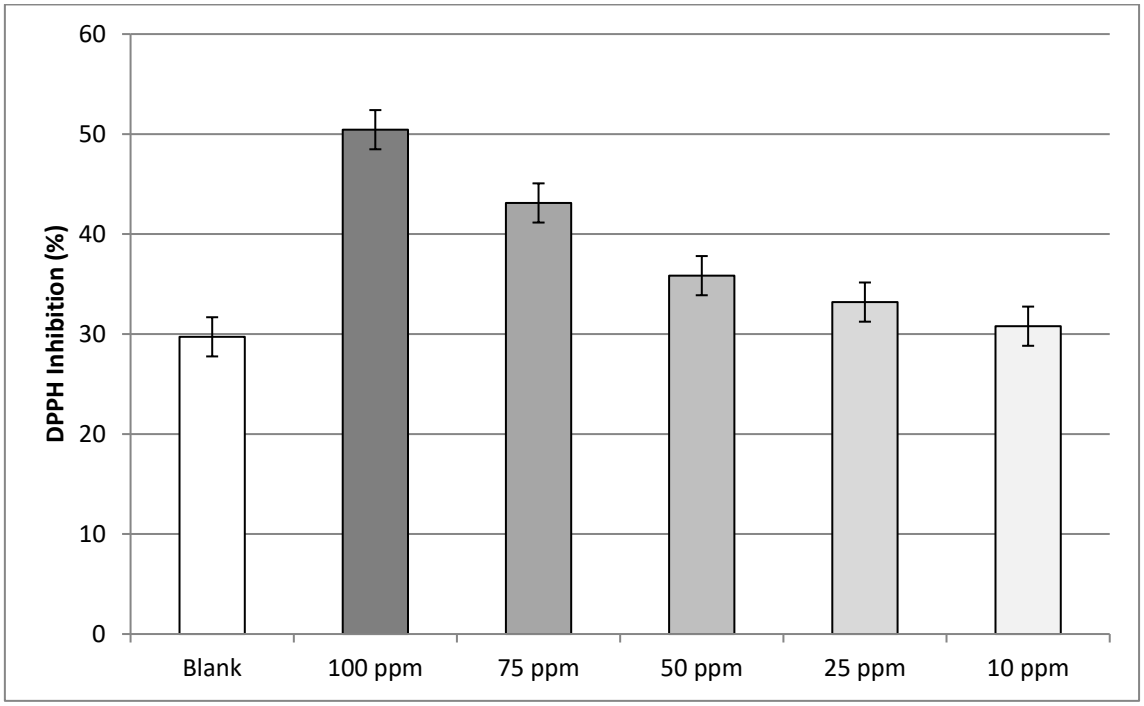


Figure 2b: Results of DPPH inhibition obtained for adhesives with different concentrations of SeNPs prepared from solution 9 after ultrafiltration.

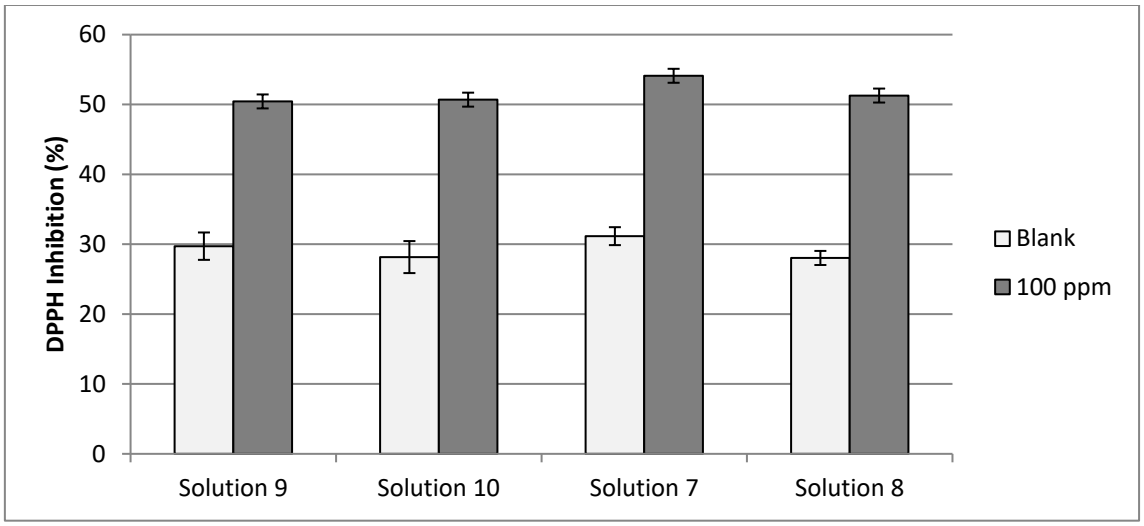


Figure 2c: Antioxidant capacity (expressed as % DPPH inhibition) for the adhesives prepared with the solutions with 2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate as stabilizing agent, after ultrafiltration.

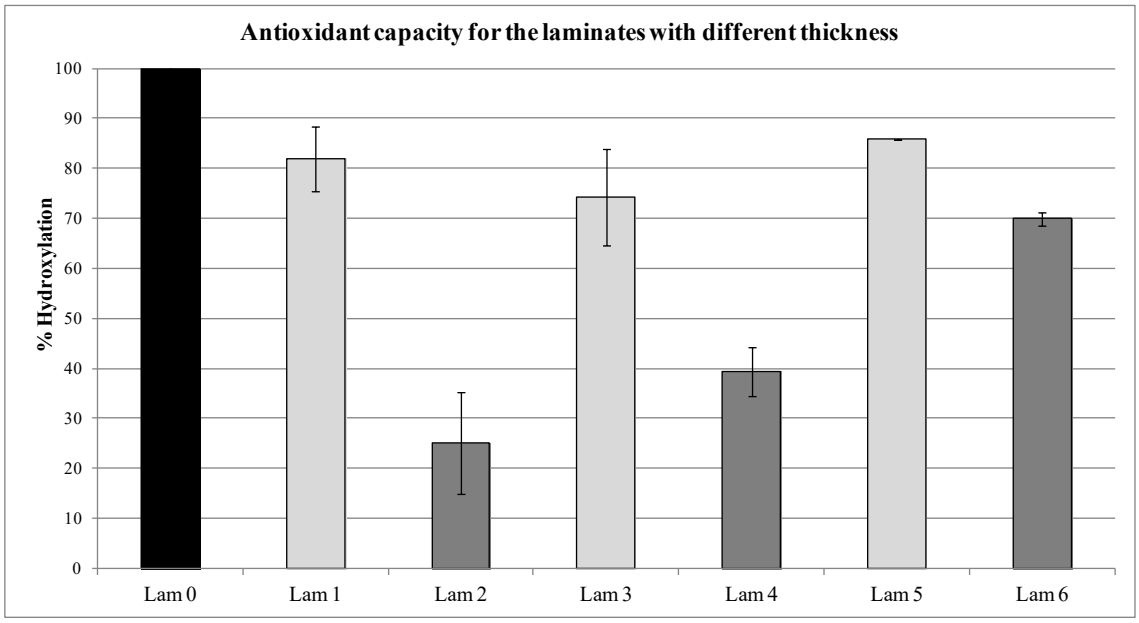
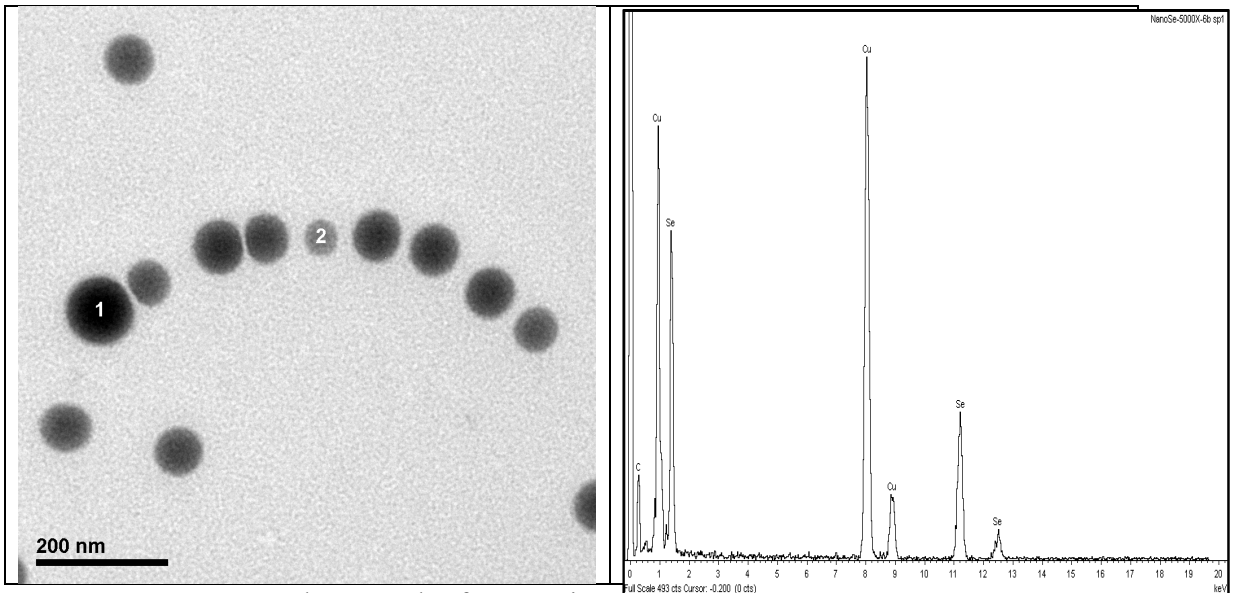
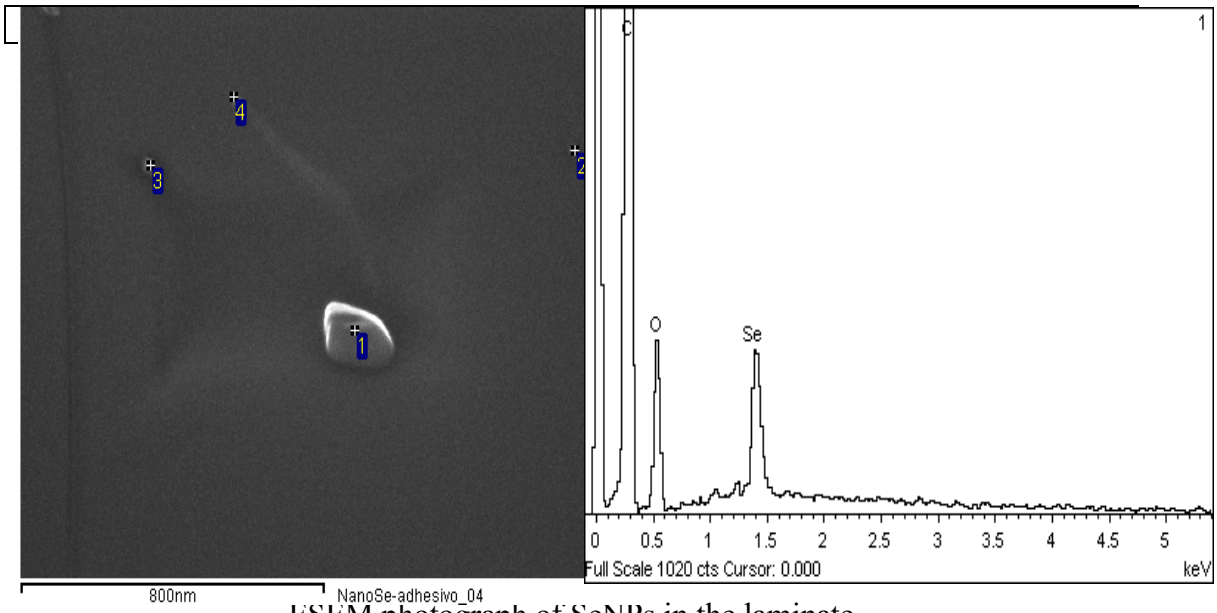


Figure 3: Antioxidant capacity expressed as % hydroxylation for the laminates with or without Se NPs for different thickness.



TEM photograph of SeNPs incorporated in the adhesive



ESEM photograph of SeNPs in the laminate

Figure 4: TEM photograph of SeNPs incorporated in the adhesive with their compositions on the top and a FESEM photograph of SeNPs in the laminate with their compositions on the bottom

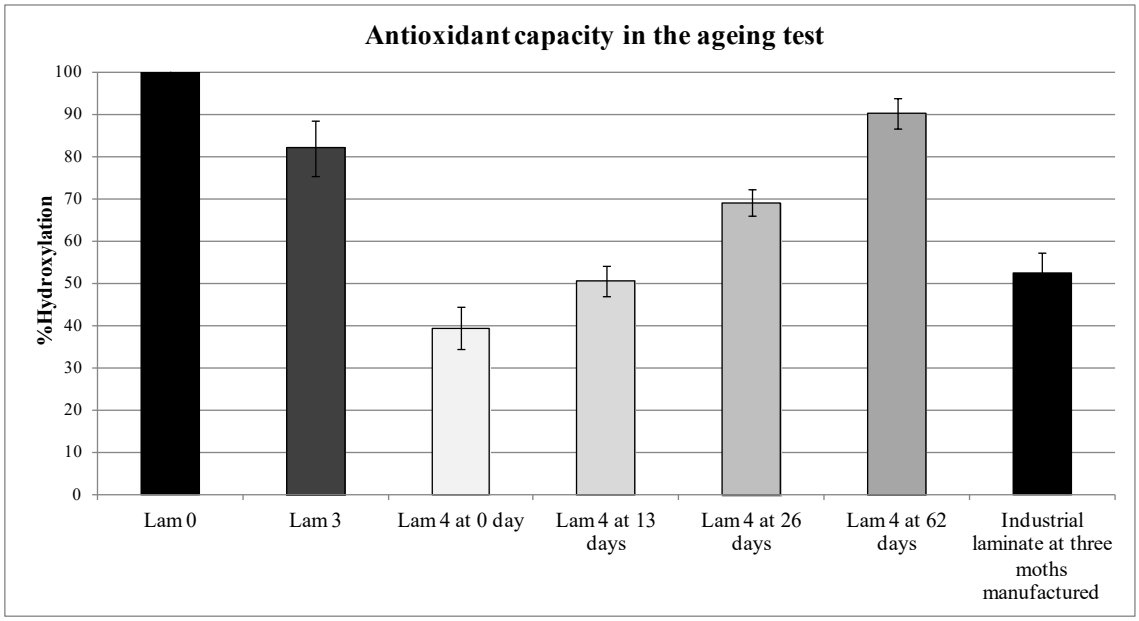


Figure 5: Antioxidant capacity expressed as % hydroxylation for the laminates with PE 60 µm in the ageing test