Asymmetrical flow field-flow fractionation coupled to inductively coupled plasma mass spectrometry for sizing SeNPs for packaging applications

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Abstract

This paper describes the application of Asymmetrical Flow Field-Flow Fractionation (AF4) coupled to diode array detector (DAD) and inductively coupled plasma mass spectrometry (AF4-UV-ICP-MS) to characterize selenium nanoparticles (SeNPs) in an aqueous acrylic adhesive to be used in a multilayer food packaging material. SeNPs were synthesized using a solution-phase approach based on the reduction of selenite with ascorbic acid in presence of different stabilizers compatiblewith food industry such as polysaccharides (chitosan (poly(D-glucosamine) and hydroxyethylcellulose (HEC)) and non-ionic surfactants (Triton X-100 (t-octylphenoxypolyethoxyethanol), 2,4,7,9-tetramethyl 5decyne-4,7-diol ethoxylate, and isotridecanol ethoxylate). Several parameters such as pH, ascorbic acid and stabilizers concentration, and compatibility of the stabilizer with the adhesive were evaluated. SeNPs suspensionswith sphericalmorphologywere obtained exceptwhen isotridecanol ethoxylatewas employed which provides SeNPs with a nanorod morphology. AF4-DAD-ICP-MS was further applied for sizing the different SeNPs preparations. DAD was used as detector for selecting the best AF4 separation conditions before coupling to ICP-MS to ensure unequivocal identification of NPs. AF4 calibration with polystyrene latex (PSL) beads of known sizes allowed size determination of the different SeNPs. The following estimated hydrodynamic sizes (expressed as the mean ± standard deviation, n = 6 replicates) were found: chitosan-SeNPs- (26 ± 3 nm), TritonX100-SeNPs (22 ± 10 nm) HEC- SeNPs (91 ± 8 nm) and 2,4,7,9-tetramethyl 5decyne-4,7-diol ethoxylate- SeNPs (59±4nm). The proposedmethodologywas successfully applied to the characterizationinterms of size of aqueous acrylic adhesives containing SeNPs Results from AF4-ICP-MS and TEM shown that only those SeNPs obtainedwith non-ionic surfactants and HECwere compatible with the adhesive. The results reported here evidence the usefulness of AF4-ICP-MS as analytical tool for controlling thosemanufacturing process involving nanoparticles which opens new frontiers in applicability of AF4-ICP-MS.

1. Introduction

Nanotechnology is a new and fast emerging field that involves the characterization, preparation, and/ormanipulation of nanostructures, devices or materials that contain components with at least one dimension of approximately 1–100 nmin length [1]. Several potential uses of nanotechnology in various segments of the food industry have been identified, such as agriculture (e.g., release of pesticides and fertilizers; animal and plant pathogen detection), food processing (e.g., nanoencapsulation of flavor or aroma

compounds; gelation and viscosifying agents), food packaging (e.g., UV protection, high barrier increase to gases, antimicrobials) and dietary supplements (e.g., mineral and vitamin fortification). But without a doubt packaging materials is one of the most important applications in the food area [2]. Besides preserving food from oxygen, light, pathogenic microorganisms, etc., food packages have many other functions, and their characteristics may affect the shelf life of food [3]. Polymers are quite frequently used for these purposes, due to their advantages over conventional materials (e.g., metal, ceramic (glass), and paper). One approach to improve the barrier properties of polymers is the incorporation of nanoparticles (NPs) (e.g., silicate NPs, silver NPs, nanocomposites) into the food packaging material, either for direct or indirect contact with the food. SeNPs act as free radical scavengers and so they could be used in indirect contact in food packaging. One clever idea is to incorporate SeNPs together with the adhesive in a multilayer structure [4]. Most of multilayer structures are built using adhesive to glue the different films. One common problem when working with NPs is their tendency to agglomerate to form clusters, which change the properties with respect to those of the dispersed NPs. Packaging materials are industrially produced, and usually require quite long storage of the raw matters used to build the materials. This storage could affect the initial properties of nanoparticles. Therefore, the development of an analytical methodology for characterizingNPs is a key pointwhen preparing packagingmaterials with NPs as additives. Electron microscopy techniques (TEM or SEM) have been widely used for the size and morphology characterization of NPs. In the last few years, the combination of separation techniques (mainly field flow fractionation, FFF and hydrodynamic chromatography, HDC) with detection techniques (ultraviolet-visible spectroscopy, UV–VIS, dynamic light scattering, DLS or inductively coupled plasma mass spectrometry, ICP-MS) have appeared as alternative to electron microscopy techniques specially for the hydrodynamic size measurements in suspensions containing a wide size ranges (polydispersions). Furthermore, these techniques allow direct size evaluation of NPs in their native dispersions, avoiding possible artifacts derived from sample preparation as itmight happen in electron microscopy analysis. Among the techniques applicable to the detection and elemental characterization of NPS the use of Asymmetrical Flow Field-Flow Fractionation (AF4) coupled to the inductively coupled plasma-mass spectrometry (ICP-MS) appears extremely promising and applicable to a number of samples from environmental to biological samples. This technique combines the ability of AF4 for sizingwith the high sensitivity and element specificity of ICP-MS, thus providing an ideal

tool for the simultaneous detection and quantification. In spite of this, the application of AF4-ICP-MS to real samples is quite scarce. In this paper AF4-ICP-MS is used for first time for controlling the integrity of SeNPs (in terms of size and composition) during the manufacture of a product (food packaging) containing SeNPs as antioxidant additive. It should keep in mind that the properties of the final product (antioxidant activity) are dependent on the properties of nanoparticles, therefore the manufacturing process should not alter the nanoparticles either in their size or in their composition. To obtain SeNPs compatible with the food packaging, the synthesis was performed in presence of either a polysaccharide, (e.g., chitosan, a poly(D-glucosamine), hydroxyethylcellulose (HEC)) or ethoxylated non-ionic surfactants (Triton X-100 (t-octylphenoxypolyethoxy-ethanol), 2,4,7,9-tetramethyl-5decyne-4,7-diol ethoxylate and isotridecanol ethoxylate). The results obtained by AF4-ICP-MS were compared with those provided by transmission electron microscopy (TEM).

2. Material and methods

2.1. Instrumentation

A 1000 W MSP microwave oven (CEM, Matthews, NC, USA) equipped with temperature and pressure feedback controllers and 12 high-pressure vessels of 100mL inner volume, operating at amaximum power of 1600Wwas used for microwave acid digestion. An Agilent 7700x collision/reaction cell ICP-MS (using H2 collision gas) (Agilent Technologies, Santa Clara, CA, USA) fittedwith aMeinhard nebulizer and an impact bead quartz spray chamber was employed for selenium detection under the operating conditions given in Table 1. An Asymmetrical Flow Field-Flow Fractionation (AF4) AF 2000 MT (Postnova Analytics, Germany) coupled to a diode array detector (DAD) and ICP-MS were used for size fractionation. The AF4 channel was fitted with a regenerated cellulose membrane of 10 kDa molecular weight cut-off and a spacer of 500 μm. The optimized AF4 settings and flows used for the separations, and the DAD and ICP-MS detection operating conditions are provided in Table 1. TheAF4 system was calibrated formolecular diameter size determination by using polystyrene latex (PSL) beads reference standards of three known sizes (22, 54 and 100 nm). Themorphological characteristics of the NPswere examined using a high resolution transmission electron microscope (TEM) (JEOL J2000 and 2100, USA) equipped with an X-ray Energy Dispersive Spectroscopy (XEDS) microanalysis composition system (Oxford Inca, city). Samples for TEM analysis were prepared by evaporating a drop of SeNPs dispersion onto

a 300 mesh lacey carbon copper grid. The efficiency of SeNPs formation was assessed by controlling the percentage of ionic selenium that was not converted into NPs. Thus, ionic selenium was separated by ultra-centrifugation (Eppendorf centrifuge 5804 F34-6-38) using 10 kDa molecular weight cut-off filters (Millipore, Ann Arbor, MI). Ionic selenium in the filtrate was measured by ICP-MS.

Table 1: Operating conditions for AF4-DAD-ICP-MS.

ICP-MS parameters	
RF Power (W)	1550
Plasma gas flow rate (L min^{-1})	15.0
Ar auxiliary flow rate (L min^{-1})	0.30
Carrier gas flow rate (L min^{-1})	0.75
Nebulizer	Meinhard
Spray Chamber	Scott
Acquisition mode	Continuous
Isotopes monitored	^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se
Replicates	3
Reaction gas	H_2
Reaction gas (mL H_2 min^{-1})	6
AF^4 separation parameters	
Membrane	Cellulose regenerated (10 kDa cut-off filter)
Spacer (µm)	500
Mobile phases	H_2O (pH 5.0)
Injection flow (mL min^{-1})	0.1
Injection time (min)	2
Cross-flow (mL min^{-1})	2
Detector flow (mL min^{-1})	1
Gradient mode	Linear
Injection volume (µL)	200
Wavelength (nm)	369

2.2. Standards and reagents

All solvents/chemicals used were of analytical grade. All solutions were prepared with deionized Milli-Q water (Millipore, Bedford, MA). The inorganic Se solution was obtained by dissolving Na2SeO3 (Merck, Darmstadt, Germany) in deionized water. The stabilizing agents used for the synthesis were chitosan, a polysaccharide derived from shrimp shells (340 gmol−1MWand ≥75% deacetylation degree); the following non-ionic surfactants: Triton X-100 from Fluka, 2,4,7,9-tetramethyl 5decyne-4,7-diol ethoxylate from Sigma, and iso-tridecanol ethoxylate (Sasol, South Africa) and a polymer such as hydroxyethylcellulose from Sigma. Ascorbic acid was obtained from Sigma-Aldrich. Acetic acid was purchased from Scharlab. Chitosan solutions of different concentrations were prepared by dissolving chitosan in 3% (w/v) acetic acid solution and shaking the mixture until a transparent solution was obtained. The remaining stabilizing agents were

diluted with Milli-Q water (Millipore, Bedford, MA) to achieve the desired concentrations.

All solutions were filtered through 0.45 μm nylon filters. A waterbased polyurethane adhesive was used as vehicle to incorporate the SeNPs into the multilayer packaging.

2.3. Synthesis of selenium nanoparticles

SeNPs were synthesized in the laboratory through the reduction of selenite with ascorbic acid using different stabilizers (chitosan (poly(D-glucosamine), hydroxyethylcellulose, Triton X-100 (toctylphenoxypolyethoxyethanol), 2,4,7,9-tetramethyl 5decyne-4,7- diol ethoxylate, and iso-tridecanol ethoxylate. First, the solution containing the stabilizing agentwas dropwisemixed with the reducing agent and stirred for 30min at room temperature usingmagnetic agitation. Next, the selenite solution (from 100 to 1000 mg L−1) was slowly added to the above solution and stirred for 30 min at room temperature. Different stabilizing agents/ascorbic acid and ascorbic acid/Na2SeO3 weight ratio were tested. Finally, the SeNPs suspensions were submitted to a dialysis process with the aim of removing the excess of reagents used for SeNPs synthesis. Dialysis was performed during 24 h at room temperature against Milli-Q water using dialysis membranes with a molecular mass cut-off of 3.5 kDa. The water used in the dialysis process was changed twice over the 24 h of the experiment.

2.4. Efficiency of the selenium nanoparticles synthesis

The efficiency of nanoparticles formation was calculated by ICP-MS measurements, after a mass balance of the amount of selenium added and the amount of free selenium obtained after filtering the suspension with a 10 kDa molecular weight cut off filter by applying centrifugation at 4000g for 30 min at room temperature and following the equation given below:

%Efficiency of NP formation ¼ Seleniumtotal− Seleniumfree Seleniumtotal _ 100

Total seleniumdetermination in the filtrate (free selenium)was performed by ICP-MS aftermicrowave digestion by using 500 μL of concentrated HNO3 (Merck) and 250 μL of 30% hydrogen peroxide.

2.5. Compatibility of SeNPs with adhesive

The different solutions containing SeNPs were added to several aqueous dispersions ofwater-based polyurethane adhesive. The formulas used cannot be disclosed for

confidentiality reasons. The technical performance of the final adhesive containing SeNPs was studied. The presence and size of SeNPs in the final adhesive was evaluated by TEM and AF4-ICP-MS.

3. Results and discussion

3.1. Preparation of SeNPs using different stabilizers.

Characterization by transmission electron microscopy A first batch of SeNPs was prepared based on the reduction of selenite with ascorbic acid using chitosan as stabilizer. The capping of SeNPs with chitosan occurs through an electrostatic interaction between the protonated form of –CH-NH3 + and ascorbic acid, which results in surface charge reduction [5]. Several reports have documented the mucoadhesive properties [6,7] of chitosan due to molecular attractive forces caused by positively charged chitosan and negatively charged mucosal surfaces. Besides these advantages in terms of SeNPs stabilization, chitosan -based materials are of interest in food industry because chitosan is a polysaccharide with natural antimicrobial properties, adding an antimicrobial effect to the antioxidant effect of SeNPs [8]. A second generation of SeNPs was prepared by following a similar procedure to the one described above but using non-ionic surfactants as stabilizers. Non-ionic surfactants have several advantages over polysaccharides [9] for the stabilization of NPs such as high hydrophobicity, formation of stable emulsions, low toxicity and low pH – sensitivity [10,11]. However, these compounds are more sensitive to temperature changes. Non-ionic surfactants are adsorbed to NP surfaces by either a hydrophilic or a hydrophobic group oriented towards the surface. The adsorption process depends on the polarity of the surface. Several ethoxylated non-ionic surfactants (Triton X-100 (t-octylphenoxypolyethoxyethanol), 2,4,7,9- tetramethyl 5decyne-4,7-diol ethoxylate, and isotridecanol ethoxylate were tested. Finally, a third batch SeNPs was prepared using hydroxyethylcellulose (HEC) as stabilizer agent. The reason of selecting this polysaccharide is its high applicability in food industry. Fig. 1 shows the chemical structure of the different stabilizing agents tested. The following parameters affecting SeNPs preparation were optimized: stabilizing agent (0.01 to 0.5%,(m/v), selenite (0.054 M) and ascorbic acid (0.027 M, 0.054 M, 0.108 M, 0.27 M and 0.34 M) concentration. The best conditions were obtained by applying a concentration of 0.1% (m/v) of stabilizer, a concentration of 0.054Mof ascorbic acid, a concentration of 0.054 M of selenite and a pH value of 3.0. Under these conditions, the efficiency of SeNPs formation, measured as indicated in section 2.4 was close to 100% (91 ± 6%) as the concentration of selenium

measured in the filtrate was almost negligible. As it has been previously mentioned, the resulting SeNPs suspensions were submitted to a dialysis process with the aim of removing the excess of reagents used for SeNPs synthesis. Seleniumconcentration was measured in the dialyzated solution by ICP-MS. For this purpose, the solutionwas previously microwave digested by using 500 μL of concentrated HNO3 (Merck) and 250 μL of 30% hydrogen peroxide. The concentration of selenium found in the dialyzated solution was below limit of detection (0.1 ppb) showing the good performance of the dialysis process for removing the excess of reagents while keeping SeNPs in the solution. The different stabilizers led to morphological differences in the resulting SeNPs as it can be seen in the TEM micrographs in Fig. 2. SeNPs generated in the presence of poly(D-glucosamine) (chitosan), toctylphenoxypolyethoxyethanol (Triton X-100), 2,4,7,9-tetramethyl 5decyne-4,7-diol ethoxylate and hydroxyethylcellulose are spherical and well dispersed. Their diameter size ranged between 18 and 100 nm. In contrast, SeNPs generated from iso-tridecanol ethoxylate produced nanorods with variable thickness and a length of 4.8 ± 0.9 μmas shown in Fig. 2d. The presence of seleniumin these nanoparticles was confirmed by XEDS which spectra show the presence of Se emission peaks consisting of SeLα, SeKα and SeKβ at 1.4, 11.22 and 12.49 keV. Stabilizer and reducing agent concentrationwere the two experimental parameters affecting SeNPs size. NPs size grows linearly with increasing stabilizer concentration in all tested cases, except for those generated with iso-tridecanol ethoxylate for which its effect of SeNPs size was not possible to determine because of the morphology of the nano-rods of the resulting NPs. In contrast, a change in concentration of HEC (from0.1 to 0.5%) did not affect the size SeNPs (Fig. S1a (Appendix)). Concerning to ascorbic acid concentration, it was found that size of particles increases as a result of decreasing the relative concentrations of the reducing agent (Fig. S1b (Appendix)). However, the differences in size are not as significant as those observed when evaluating stabilizing concentration. Table 2 compiles the average and uncertainty values of the SeNPs sizes provide by TEM.

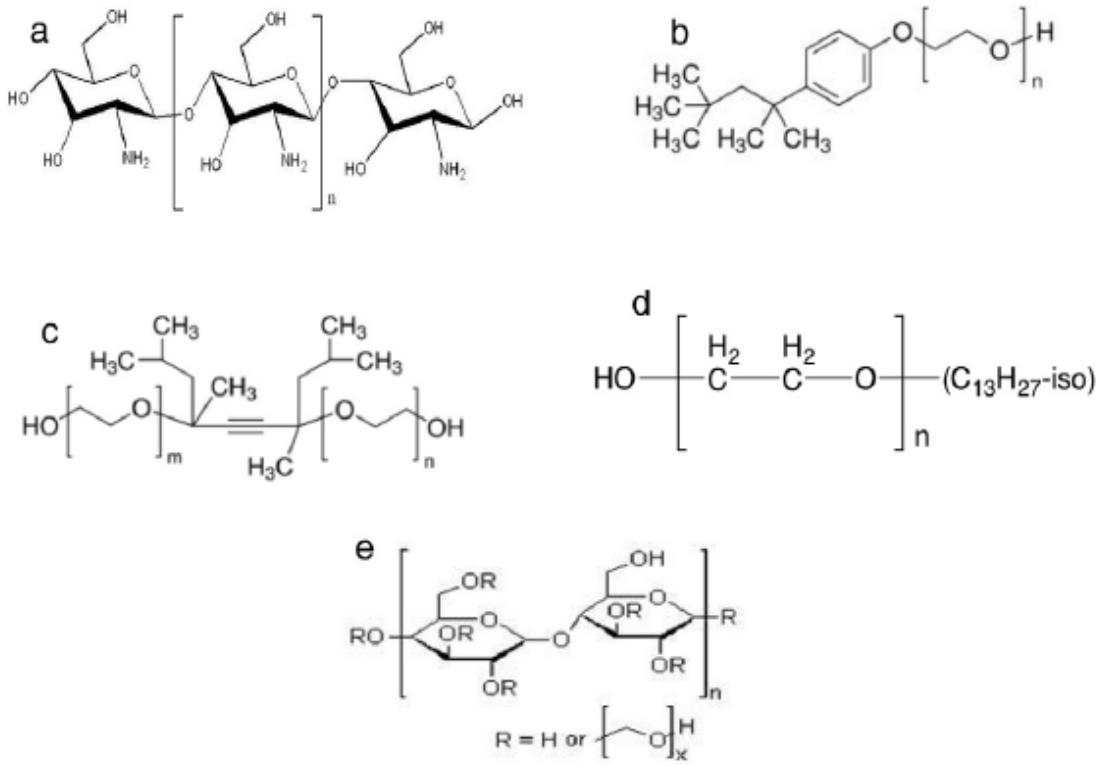

Fig. 1. Chemical structure of stabilizing agents: a) poly(D-glucosamine) (chitosan), b) t-octylphenoxypolyethoxyethanol (Triton X100), c) 2,4,7,9-tetramethyl 5decyne-4,7-diol ethoxylate, d) isotridecanol ethoxylate and e) hydroxyethylcellulose.

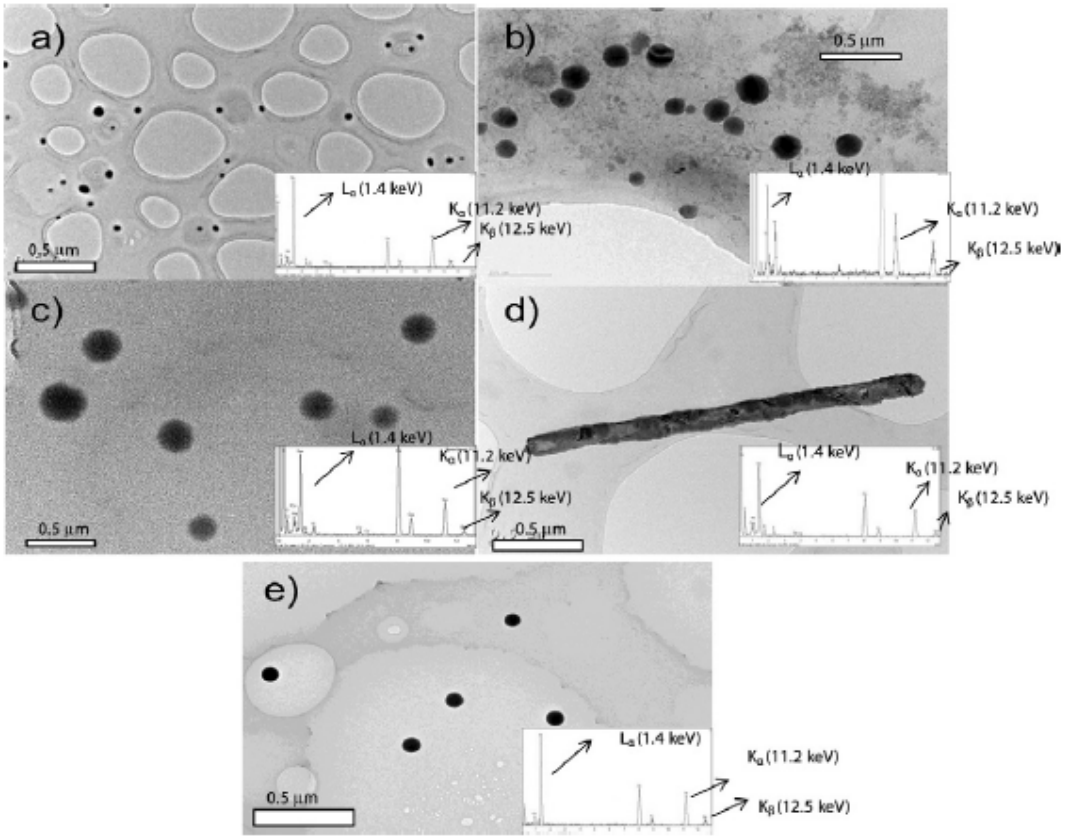

Fig. 2. TEM images and XEDS spectra of SeNPs solutions at pH 5, T = 20 ± 1 °C, 0.1% stabilizing agent, 0.054 M ascorbic acid and 10 μg g−1 Se. a) (D-glucosamine) (chitosan), b) toctylphenoxypolyethoxyethanol (Triton X100), c) 2,4,7,9-tetramethyl 5 decyne-4,7-diol ethoxylate, d) iso-tridecanol ethoxylate and e) hydroxyethylcellulose.

Table 2: SeNPs sizes provide by TEM and AF4-DAD-ICP-MS.

SeNPs	TEM (nm)[a]	AF4-DAD-ICPMS (nm)[b]
Chitosan	25 ± 5 nm	26 ± 3 nm
TritonX100	23 ± 3 nm	22 ± 10 nm
Ethoxylate	53 ± 6 nm	59 ± 4 nm
Hydroxyethycellullose	101 ± 6 nm	91 ± 8 nm

[a] TEM measurements: Results expressed as the mean value based on the diameter of about a hundred particles.
[b] AF4-DAD-ICP-MS: Results expressed as the mean value ± standard deviation (n = 6 replicates).

3.2. Characterization of SeNPs by AF4-DAD-ICP-MS

In this work, the capability of AF4 on line coupled to UV and ICP-MS was evaluated for determining the SeNPs size as an alternative to TEM measurements. AF4 is a single-phase separation technique in which

the separation is achieved within a very thin channel, against which a perpendicular force field is applied. The coupling of AF4 to ICP-MS provides detection limits (LOD) formetallic NPs three orders of magnitude lower than other detector systems such as Dynamic Light Scattering (DLS) or Multiangle Light Scattering (MALS), allowing the unequivocal identification of NPs. In the current work, UV–VIS spectrometry was employed as AF4 detector as a first step for selecting the best separation conditions in a rapid and cheap manner compared to the use of ICP-MS. Once AF4 separation was optimized, the systemwas on line coupled to the ICP-MS to ensure unequivocal identification of NPs. DADmeasurementswere carried out at awavelength of 369 nm. Thiswavelengthwas selected by recording the UV–VIS spectra of different SeNPs dispersions. The following parameters affecting AF4 performance for SeNPs separation such as mobile phase composition, flow-rates and channel dimensions were optimized: The criteria employed for selecting AF4 separation conditions was based on two requirements: a good resolution in fractionation and a quantitative recovery of the fractionation method. Cross-flow is one of the most important parameters affecting NPs separation. The cross flow was evaluated from 0 to 4 mL min−1. Best resultswere attainedwhen using a cross flow of 4mLmin−1, however, the use of this high flowproduced a high retention time alongwith a dilution of the sample.With the aimof decreasing dilutionwhile keeping optimum separation resolution, the spacer thickness was modified from 250 μm to 500 μm. FFF theory predicts a direct dependency on elution

time on spacer thickness so an increasing in retention time is expected for thicker spacer and therefore, a better resolution in separation. For instance, increasing the spacer by a factor of two has the same effect in retention time as increasing the cross flowby a factor of four. Thus the use of a spacer of 500 μmallowed us to decrease the cross-flowrate at a value of 2 mL min−1 and therefore decreasing dilution while keeping optimum resolution. The pH value directly influences the zeta potential of nanoparticles and this may influence not only NPs stability but also the interactions with the permeation membrane. The ideal situation is to select a pH that provides the highest repulsion between the NPs themselves and with the membrane to avoid aggregation or adsorption phenomena. In the current work, the SeNPs are stabilized with polymers which could suffer aggregation phenomena as pH increases, thus a mobile phase adjusted at pH 5.0 was used for further experiments. The optimized AF4 settings and flows used for the nanoparticles fractionation and the DAD and ICP-MS detection operating conditions are detailed in Table 1. Fractograms were obtained after injecting 200 μL of the SeNPs suspension using a constant cross-flow of 2 mL min−1 for the first 2 min, a linear gradient 2–0 mL for the subsequent 20 min, and then 0 mL min−1 for the last 10 min. The use of water as mobile phase is also of importance with respect to other AF4 separation procedures using surfactants as mobile phases. The use of surfactants could lead to either a surfacemodification of NPs or to an irreversible interaction of NPswith the channelmembrane. The fractograms in Fig. 3A, B, C and D show the fractionation of SeNPs prepared in chitosan, Triton X-100, ethoxylated 2,4,7,9-tetramethyl 5decyne-4,7-diol and hydroxyetylcellulose, respectively. Se-containing peaks were identified by ICP-MS. Two peaks are observed in all fractograms. The first one could be considered as unfocused species eluted at the void time and therefore, will not be considered for sizing purposes. The second peak appears at different retention time depending on the type of stabilizer used suggesting differentNPs sizeswhich is in agreementwith those results previously obtained by using TEM. By applying the selected separation conditions, a good separation was obtained between the void peak and the second peak, except in case of Triton X-100 (Fig. 3B) where the second peak is unresolved. In spite of the poor resolution obtained when separating SeNPs modified with Triton X-100, the optimized separation conditions were applied for further studies since it allowed us the fractionation of most SeNPs employed in this study. The profiles and tailings ofmost of the peaks in the fractograms suggest the presence of polydisperse systems composed with spherical nanoparticles of different sizes which is an agreement with the results obtained by

applying TEM. The performance of the fractionation method (the ability of the method to fractionate analytes without a substantial loss of material) was assessed by calculating the recovery (R (%)), which is defined as the ratio between recovered mass after analysis, m, and initial injected mass, mo.

R (%)= (m/mo) x 100

The recovery was calculated following an on-line approach where the sample is injected both with and without applying a cross-flow field. In this case, the area under each peak is integrated, and the difference represents the analyte mass loss in the channel [12]. By applying the mentioned separation conditions a recovery of around 91 ± 6% was obtained for all the SeNPs tested.

Once the parameters affecting the separation were assessed and the optimal conditions established, the size diameter of the different SeNPs were measured by AF4-ICP-MS. Two different approaches can be used to determine the hydrodynamic diameter of fractionated particles, either applying the standard FFF theory or by using certified standards in size to calibrate the retention time-size relationship for a specific set of flow conditions. Both approaches consider that size fractionation is only dependent on the size of the component but independent of its chemistry. However, erroneous information may still be obtained since these calibration methods do not take into account elution time changes due to specificNP-membrane interactions, different behavior between the NPs used for calibration and the analyte, formation of aggregates and nature of nanoparticles. A common practice for size calibration is the use of well characterized, commercially available polymer (typically polystyrene spheres). Recently, it has been demonstrated that polystyrene latex beads (PLS) are suitable for sizing SeNPs [12–13]. Based on that, a size calibration procedure using polystyrene latex beads reference standards of three known sizes (22, 54 and 100nm)was employed. For this purpose, PLS standards were separated by applying the AF4 experimental separation conditions previously optimized for SeNPs. The equation obtained for the calibration was: diameter (nm) = 2.073 tr(min) + 9.893 and the correlation coefficient (r) was 0.9998. The equationwas applied for sizing the different SeNPs by interpolating the retention time of the second peak of each fractogramonto the calibration curve. The following estimated hydrodynamic sizes (expressed as the mean ± standard deviation, n = 3 replicates) were found: chitosan-SeNPs- (26 ± 3 nm), TritonX100-SeNPs (22 ± 10 nm) and HEC- SeNPs (91 ± 8 nm) and 2,4,7,9-tetramethyl 5decyne-4,7-diol ethoxylate- SeNPs (59 ± 4 nm).As it was expected TritonX100-SeNPs provide the biggest dispersion in results. The good correlation found between the results

provided by AF4 and TEM measurements (Table 2) may be explained by several factors: sphericity of SeNPs, stability of the resulting suspensions and the similarity in the Van derWaals forces provided by the SeNPs and PSL.

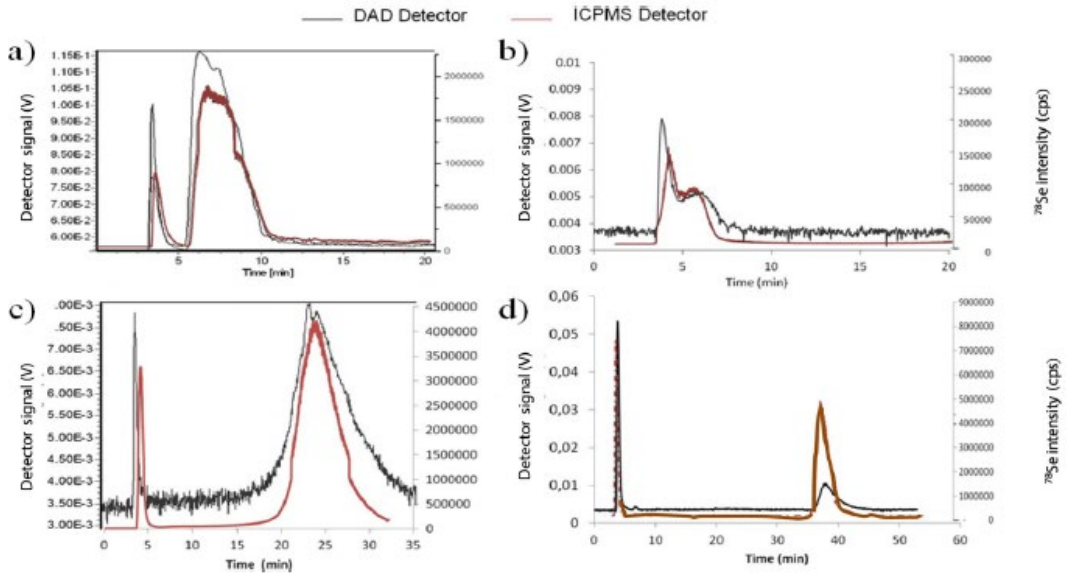

Fig. 3. AF4-DAD-ICP-MS fractograms corresponding to SeNPs solutions prepared in presence of 0.054Mascorbic acid, 10 μgmL−1 Se, pH 3.0 and by using the following stabilizers: A) 0.1% poly(D-glucosamine) (chitosan), B) t-octylphenoxypolyethoxyethanol (Triton X100), C) 0.5% ethoxylated 2,4,7,9-tetramethyl 5decyne-4,7-diol, and D) 0.5% hydroxyethylcellulose. Red line corresponds to the use of a UV detector and black line to the use of ICP-MS as detector.

3.3. Application of SeNPs to food packaging

As it has been previously mentioned, one of themost active areas of application of nanotechnology in the food sector is food packaging, Different types of metallic nanoparticles, such as AgNPs and TiO2 NPs, incorporated into food contact polymers to enhance mechanical and barrier properties, have been proposed [14–16] but all of themare in direct contactwith the food.However, according to the EU legislation [17] the use of NPs in direct contact with food is only permitted when using titaniumnitride. Other NPs have to be evaluated case by case, as there is not information about the toxicity of NPs because of their size.

To avoid the direct contact, NPs could be incorporated behind the polymeric layer in contact with food. Two key points have to be considered in this case: First, the NPs have to act without direct contact and second, a suitable technology for incorporating the NPs has to be selected. Thus, one clever solution is to use adhesives as vehicle to incorporate the NPs in a multilayer packaging material. This technology has been successfully

employed to produce antioxidant packaging materials [18–19,4]. Selenium nanoparticles (SeNPs) have shown antioxidant properties which made them good candidates for building antioxidant packaging materials to extend the shelf life of food. In the current work, three types of stable SeNPs suspensions were synthesized and fully characterized, however for being used in multilayer packaging, SeNPs need to be compatible with the adhesive. Therefore, the NPs dispersion was incorporated into the packaging material using waterbased polyurethane adhesive. Due to the fact that the water-based adhesive has an anionic character, aggregation of SeNPs took place when chitosan was used as stabilizer. In contrast, the use of SeNPs coated with a non-ionic surfactant and hydroxyethylcellulose were initially compatible with the adhesive tested. Thus, one of the most remarkable advantages of using non-ionic surfactants is that they can be usedwithin a higher pH rangewithout affecting the characteristics of the NPs (including their size), which is of great relevance if they are going to be used in food packaging. Once chitosanwas rejected, the compatibility of SeNPs in the surfactants and in hydroxyethylcellulose with the adhesive was evaluated. The size of SeNPs once incorporated into the adhesive was evaluated by TEM analysis and no morphological changes were observed. Moreover, no nanoparticles aggregation was observed when preparing the adhesive. Thus, all the above synthesized SeNPs (except chitosan) are suitable for further application in food packaging. The performance of AF4-ICP-MS for sizing SeNPs once incorporated into the adhesive was also tested. For this purpose, the adhesive containing SeNPs was diluted in water and further analyzed by AF4-ICP-MS following the conditions given in Table 1. Size of the SeNPs was estimated by using the size calibration equation given above. As an example, the fractogram obtained for the adhesive containing SeNPs (prepared in hydroxyethylcellulose) diluted inwater is shown in Fig. 4. Thefractogram shows two peaks, at a retention time of corresponding to the void volume and 42 min corresponding to an estimated diameter (based on polystyrene latex bead reference standard retention time) of 97 nm. A shift in the retention time of the second peak is observed when comparing fractograms in presence (Fig. 3D, 38 min) and in absence (Fig. 4, 42 min) of adhesive. However in terms of SeNPs sizes, the differences are not so relevant and they can be consideredwithin standard deviation (n= 3 replicates) 91 ± 6 nm and 95 ± 9 in absence and in presence of adhesive. The results are in agreement with those provided by TEM which confirms the stability of the SeNPs in the adhesive. The results obtained evidence the applicability of AF4-ICP-MS technique to size and

characterize NPs in manufacturing process. The final dispersion was homogeneous and did not show apparent problems. The adhesive-containing SeNPs formula, developed in this case for laminates in which plastic-plastic was glued, is under Patent (PCT/ES2006/000311). Thus, the formula cannot be revealed here. After preparation the adhesives containing SeNPs were left stand for one month. The adhesion properties of the adhesive containing SeNPs after one month of storage was the same as that recently prepared, what confirms the stability of the adhesive during one month.

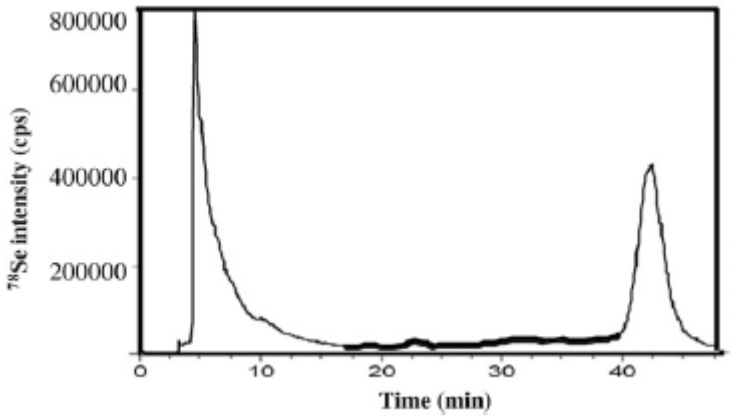

Fig. 4. AF4-ICP-MS fractogram corresponding to SeNPs-containing adhesive diluted in water.

4. Conclusions

AF4-ICP-MS has been shown as a useful analytical tool for sizing SeNPs, either in solution or in an aqueous adhesive. The developed analytical methodology allows us to monitor the modifications of SeNPs once applied to adhesive for preparing multilayer packaging material with antioxidant properties. These results are of relevance since the final product is highly dependent on nanoparticles characteristics and, therefore the manufacturing process should not alter the nanoparticles either in their size or in their composition. In this line, AF4-ICP-MS is a powerful analytical tool for controlling manufacture processes when workingwith nanoparticles and, therefore it could be considered as an alternative to other most costly techniques such as TEM or light dispersion techniques. In this paper, non-ionic surfactants and sydroxyethylcellulose were used as first time as stabilizer of SeNPs. In all cases stable and spherical SeNPs compatible with the adhesive were obtained.

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