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Potentialities of polymeric electrospun membranes decorated with silver nanoparticles and graphene oxide for biodetection by SERS

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Abstract

Electrospun nanofibres are highly attractive products taking advantage of their remarkable specific surface area, high porosity and good transport properties that are ideal requirements for efficient molecule detection. In this work, fibres of polyacrylonitrile (PAN) were first prepared by electrospinning. These fibres were then coated with silver nanoparticles or silver/graphene nanocomposites (fillers) following two different methodologies. The fibres were used without and with a pre-functionalization treatment with hydroxylamine. With this functionalization, amidoxime groups at PAN fibres surface are expected to promote the linkage with the fillers. Both methodologies resulted in a fibre network covered with the fillers. The resulting membranes were tested as substrates for the detection of probe molecules by surface-enhanced Raman scattering (SERS). The results obtained show that easy to handle SERS substrates can be prepared using the electrospun fibres.

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1. Introduction

Electrospun nanofibres are powerful tools to be applied in biosensors, although limited studies and technology transfer have been done up to now in this area. Biosensors are devices able to detect and quantify an analyte in a biological sample and to respond to biological or chemical stimuli. Specific detection of proteins characteristic of some diseases plays an important role in biochemistry and biomedical research areas. In particular, the measurement of cancer biomarkers is of major importance for early detection and high reliability prognosis of such disease. This is a great challenge for the community that is dedicated to this type of

scientific research, being the development of the interface between nanoscience and medicine one of the most interesting areas of science.

Raman spectroscopy, in particular SERS (Surface-Enhanced Raman Scattering) is a very promising approach for specialized investigation due to its ability to identify structurally a molecule with very high sensitivity [1]. The enhancement of Raman signals by SERS has been observed up to 10^6 getting the particular case of single molecule detection. This notable enhancement provides high sensitivity and offers an opportunity of development of ultra-sensitive analytical methods for chemical and biochemical detection [2]. SERS has been used as a selective and sensitive method in the detection of proteins and oligonucleotides [3].

Different types of SERS substrates have been developed involving either pure or supported nanostructured metals, mostly gold and silver. A fundamental limitation of these planar metal SERS-

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active surfaces is that the analyte molecules must rely on slow diffusion from the bulk solution to the surface to facilitate molecule-metal interactions, an essential prerequisite for SERS detection. A simpler and widely employed approach to SERS detection is to directly mix metal colloids with target analytes [1]. The metal nanoparticle aggregates containing many “hot spots” suspended in solution can present a large total surface area, thus facilitating effective interactions with analytes and generation of strong Raman scattering emission.

Interestingly, natural nanofibrillar structures such as bacterial cellulosic (BC) with silver nanoparticles can act as active SERS substrates [4] BC was used as a template to grow Ag nanoparticles. The high interfacial area provided by Ag-BC cellulosic matrix for the deposition of Ag nanoparticles where organic ligands can adsorb, lead to strong Raman enhancement. The importance of substrate three-dimensionality for SERS was highlighted by several researchers [3,5,6].

Electrospinning is a remarkably robust and versatile approach to synthesize large-scale 3D networked nanofibre membranes with extremely high surface area-to-volume ratios, uniformity and mechanical resilience which could be used as nanostructure-frameworks to assemble hierarchical nanostructures for 3D SERS substrates [7].

Noble metal nanoparticles embedded electrospun nanofibres have been studied widely due to their applications in catalysis, antimicrobial, microelectronics, etc [8]. The SERS application of these types of structures has also raised the interest of several researchers [9-13].

For example, X. Li et al. [11] prepared electrospun PVA nanofibres embedded with Au–Ag alloy NPs. Different SERS signals of PVA/Au–Ag nanofibre could be observed with different analyte molecules (4-mercaptobenzoic acid, pyridine, and thiophenol) molecules. Interestingly, the electrospun PVA/Au–Ag nanofibre substrate showed the better SERS sensitivity in comparison with the drop-cast film. M. Cao et al. [12] also prepared PVA nanofibres with high concentration of Au NPs on the fibres and SERS spectra showed a significant Raman signal enhancement of 4-MBA molecules, which were absorbed on the surface of PVA/Au nanofibres, even under a relatively low concentration (10^{-6} mol dm⁻³). The nanofibres also showed the better SERS performance in comparison to the cast-films.

The assembling of SERS-active noble metal NPs onto the outer surfaces of the electrospun nanofibres was

also tested. For example, Ag nanoparticles were decorated on polyacrylonitrile [14] or poly(methyl methacrylate) nanofibre frameworks [15] via wet-chemistry routes, and Au nanorods were assembled on poly(2-vinyl pyridine) nanofibre-frameworks by a drop-cast method [16] or immobilized onto polycaprolactone nanofibre-frameworks via polyelectrolyte decoration.

Here, we aimed to obtain efficient and freestanding electrospun nanofibrous membranes that combine the flexible and portable properties of the polymeric membrane mats and the plasmonic activity of noble NPs with and without graphene and study its potential SERS activity.

Graphene is a promising material to be used as SERS substrate. Recently, graphene by itself was reported as a clear inducer of the enhancement of Raman signal intensity in adsorbed molecules [17]. This enhancement was attributed to charge transfer between graphene and the molecules of the analyte. Graphene and gold nanocomposites have demonstrated good results as SERS substrates [18]. The arrangement between gold NPs and graphene can attribute a unique mechanism of electronic transfer or energetic between both phases which allows the observation of SERS. Also, silver NPs were used in graphene-based nanocomposites to enhance Raman signals [14].

The assembling of previously prepared Ag NPs and Ag/graphene nanocomposites on polyacrylonitrile (PAN) electrospun mantles used as a physical support is presented in this article, together with its SERS analysis.

2. Experimental

2.1. Methods

2.1.1. Preparation of silver colloids and silver/graphene nanocomposites

The silver colloids were prepared according to two well established methods, one using sodium citrate (SC) based on the Lee and Meisel method [19], and the other using glucose (G) [20] as the reducing agents.

Graphene oxide (GO) was prepared from the chemical exfoliation of graphite flakes [21].

To prepare the AgGO nanocomposites the same methodologies for the preparation of the Ag NPs alone were followed, but in the presence of GO. During the application of this chemical process, GO is also partially reduced.

In the case of SC method, 7.5 mg of GO was added to a 5 mg of AgNO_3 dissolved in 50 mL of deionized water ($6 \times 10^{-4} \text{ mol dm}^{-3}$). The mixture was stirred for 30 min to promote the interaction of Ag^+ with GO surface. The suspension was then heated until 80°C , after which 3.760 mL of SC (85 mmol dm^{-3}) was added dropwise and kept under stirring during 1 hour. After cooling to room temperature, the suspension was washed by centrifugation (AgGO_SC nanocomposite). In the case of G method, 1.2 g of D-(+)glucose was dissolved into 30 mL of a suspension of GO (1.5 mg/mL) under sonication during 5 min. 1 mL of ammonia (25%) was added to 10 mL of AgNO_3 ($0.050 \text{ mol dm}^{-3}$, 0.0839 g, $v=10 \text{ mL}$). Both phases were mixed and sonicated during 5 min. After 24h, the suspension showed a grey precipitate, that was washed by centrifugation as well (AgGO_G nanocomposite).

2.1.2. Preparation of PAN fibres

1.4 g of PAN was dissolved in 10 mL of dimethylformamide, with stirring during $\sim 18 \text{ h}$. The experimental conditions for the electrospinning were 2000 rpm of the cylindrical collector placed at 12 cm distance from the needle, the voltage applied was 20 kV, and the feed rate was 1.2 mL/h.

2.1.3. PAN fibres imbibed in silver colloids and silver/graphene suspensions

Squares ($7 \times 7 \text{ cm}$) of the PAN membrane were cut and placed upon a filter paper inside a Buckner funnel. 5 mL of each suspension of metal nanoparticles (Ag_SC, Ag_G) and the corresponding GO nanocomposites were trapped by filtration through the PAN squares. The PAN squares were then dried in air.

2.1.4. Surface functionalization of PAN membranes

The as-prepared electrospun PAN fibrous membranes were treated with 1 mol dm^{-3} of NH_2OH aqueous solution at 70°C for 5 min; some $-\text{C}\equiv\text{N}$ groups on the surface of fibres reacted with NH_2OH , leading to the formation of $-\text{C}(\text{NH}_2)=\text{N}-\text{OH}$ groups. After the treatment, the obtained amidoxime surface-functionalized PAN (HDA-PAN) fibrous membranes were thoroughly rinsed in distilled water and dried

2.1.5. Preparation of composite membranes with the previously functionalized HAD-PAN

As schematized in Fig. 1, four different routes to prepare new materials were followed. The amidoxime functionalized PAN fibres were immersed in i) AgNPs colloidal suspension overnight (Ag-HAD-PAN); ii) AgGO suspension overnight (AgGO-HAD-PAN); iii) AgGO suspension overnight, dried and after immersed on a GO suspension previously reduced with HAD (rGO) for 24 hr, (rGO-Ag-HAD-PAN); iv) GO suspension during 24 hr, followed by Ag NPs attachment overnight.

The Ag NPs used in this approach were prepared with sodium citrate.

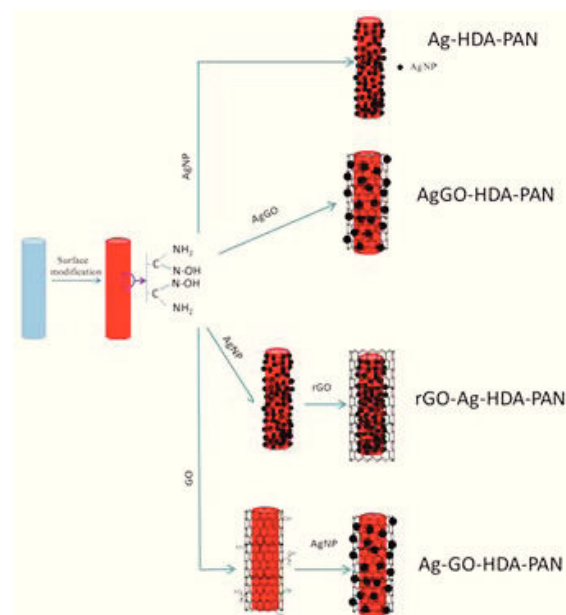


Fig. 1. Preparation scheme of four different types of materials produced with PAN functionalized: Ag-HDA-PAN, AgGO-HDA-PAN, rGO-Ag-HDA-PAN and Ag-GO-HDA-PAN

3. Results and discussion

The PAN fibres imbibed in the Ag colloids and in the Ag/GO suspensions were analysed by SEM (Figure 2). It can be observed that the nanomaterials were really entrapped in the PAN electrospun network, particularly in the case of the AgGO nanocomposites. This latter statement can be understood due to size constraints.

The SEM images show that the Ag NPs prepared with glucose are bigger and tend to agglomerate when compared with the ones produced with sodium citrate. In both cases, the distribution of Ag NPs at the GO surface seems uniform. This is important for the creation of plasmonic nanostructures which is a pre-required parameter for SERS substrates to obtain a good enhancement of the Raman signals [22]. The presence of Ag NPs on these nanocomposites was also confirmed by EDS analysis and by UV-vis spectroscopy (not shown).

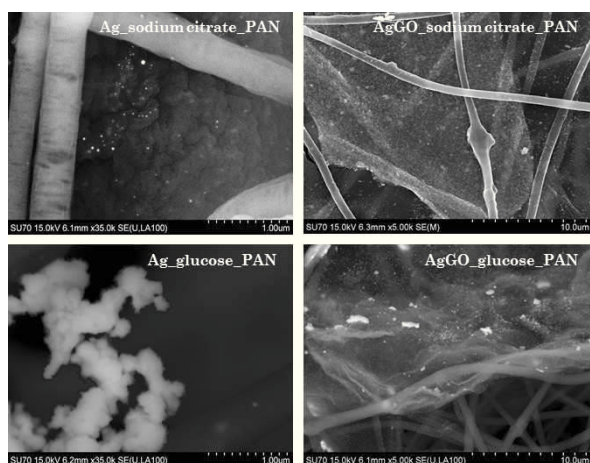


Fig. 2. SEM images of PAN fibres impregnated with Ag NPs produced with G and SC and fibres impregnated with AgGO_SC, and, AgGO_G.

The produced PAN membranes were tested for its SERS activity. Thiosalicylic acid (SHsal) ethanolic solution at 10^{-3} mol dm^{-3} concentration was the Raman probe deposited on the PAN membranes. Figure 3 shows the spectra obtained.

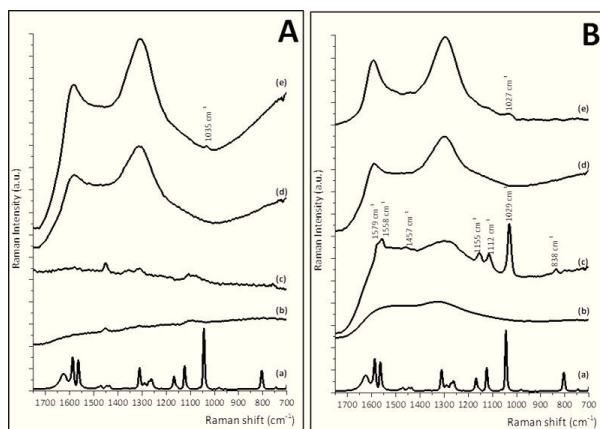


Fig. 3. A (a) SHsal reference, (b) Ag_SC_PAN, (c) Ag_SC_PAN+SHsal, (d) AgGO_SC_PAN and (e) AgGO_SC_PAN+SHsal. B- (a) SHsal reference, (b) Ag_G_PAN, (c) Ag_G_PAN+SHsal, (d) AgGO_G_PAN, (e) AgGO_G_PAN+SHsal.

For Ag_G_PAN, seven peaks with different intensities of SHsal were identified, one very strong (1029 cm^{-1}), three of medium intensity (1112 , 1155 and 1558 cm^{-1}) and three very weak (838 , 1457 and 1579 cm^{-1}). These results suggest that, under the present experimental conditions, the existence of bigger Ag NPs and agglomerates of these NPs are suitable to obtain a SERS enhancement of the probe molecule. This is in agreement with earlier reports which indicate that SERS intensity increases with increased particle size

[23,24]. The Ag_SC_PAN were not active for SERS under the present experimental conditions.

Although the intensity was weak, the typical Raman peak of SHsal around 1030 cm^{-1} was present in the spectra of both membranes prepared with AgGO. The particularity of these spectra was the clear enhancement of the main typical D and G bands of graphene [25] in the presence of the probe, independently of the preparation method (Fig. 3). The enhancement of the Raman bands of graphene observed in the composites is attributed to a SERS competitive effect of Ag NPs on the absorbed probe molecules and on graphene. This was already referred by S. Sun et al [25] in aqueous solution. Here we show that the same behaviour happens in solid substrates.

Considering now the fibres pre-functionalized with HAD. The presence of Ag NPs in the substrate Ag-HDA-PAN was confirmed by SEM (Fig. 4) and also qualitative EDS analysis confirmed the silver presence (not shown). The UV-vis absorbance shows a maximum at approximately 370 nm , which is a well-known peak position that indicates the presence of silver nanoparticles [26].

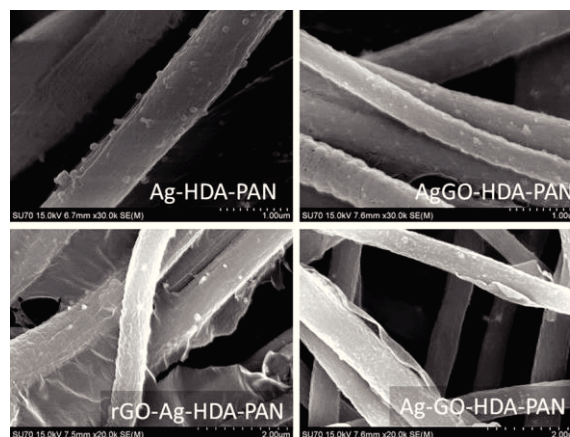


Fig. 4. SEM images from the samples Ag-HDA-PAN, AgGO-HDA-PAN, rGO-Ag-HDA-PAN, and Ag-GO-HDA-PAN

The SEM image of AgGO-HDA-PAN suggests the coating of the functionalized PAN fibres with the AgGO nanocomposites, showing a good dispersion through the fibres. The substrate rGO-Ag-HDA-PAN, clearly show the presence of rGO covering the fibres with some Ag NPs on the top. In the Ag-GO-HDA-PAN, the presence of GO sheets is also clearly observed by SEM; it seems that the GO sheets were mainly wrapped around the PAN fibres.

All the previous materials prepared were tested with Rodhamine 6G (R6G) for their SERS activity. The aqueous solution of Raman probe was drop-cast onto the surface of the substrates.

The substrate Ag-HDA-PAN showed a very good SERS spectrum for R6G at 10^{-3} mol dm^{-3} concentration, being possible to identify the peaks localized at 775, 1183, 1306, 1360, 1449, 1505, 1597, and 1643 cm^{-1} (Fig. 5). Almost the same peaks were identified at 10^{-5} mol dm^{-3} . Surprisingly, at the concentration of R6G 10^{-6} mol dm^{-3} no peak was observed.

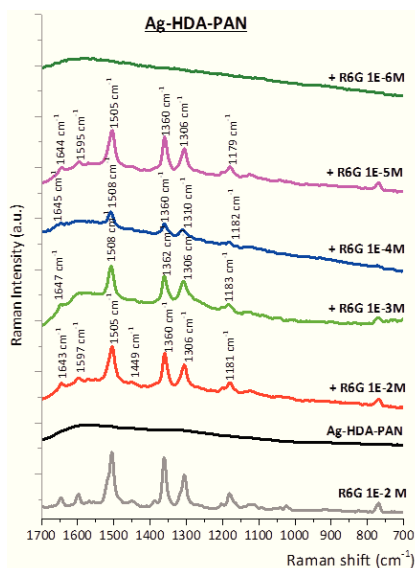


Fig. 5. Limit of detection of R6G in Ag-HDA-PAN substrate. The Raman spectra of R6G at 10^{-2} mol dm^{-3} concentration was also inserted for comparison.

When the membranes were prepared with the GO containing nanocomposites, the presence of D and G bands at 1290 and 1580 cm^{-1} are visible in all SERS spectra, like previously with the other membranes. The intensity of these bands may suppress the identification of the probe molecule, being not possible to identify the analyte for a concentration higher than 10^{-2} mol dm^{-3} (spectra not shown).

However, in the case of the Ag-GO-HAD-PAN substrate the SERS peaks of R6G showed enough enhancements to overlap the graphene bands. In this case, we prepared several membranes with different Ag to GO molar ratios, which were Ag-0.18GO, Ag-0.09GO and Ag-0.06GO. The substrate prepared with Ag-0.09GO showed the best enhancement with R6G at 10^{-3} mol dm^{-3} and also a small peak was visualized at 10^{-4} mol dm^{-3} (Fig. 6).

Comparing the four routes tested with the pre-functionalization of PAN fibres, it seems that the

presence of Ag NPs at the exterior surface contributes for the SERS enhancement, which is the case of Ag-HAD-PAN and Ag-GO-HAD-PAN. The presence of GO or reduced GO as an outer layer is not effective, since it may cover the Ag NPs and inhibit the SERS effect.

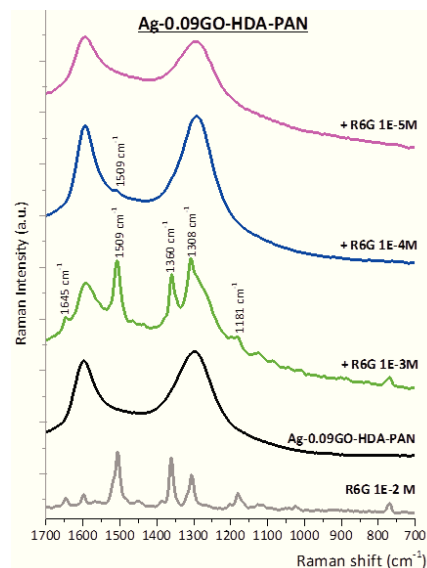


Fig. 6. SERS spectra of R6G in several concentrations using Ag-0.09GO-HDA-PAN. The Raman spectrum of R6G at 10^{-2} mol dm^{-3} concentration was also inserted for comparison.

Also the reduction degree of the GO nanosheets can be interfering. It is referred that mildly reduced GO nanosheets can significantly increase the chemical enhancement of the main peaks by up to 1 order of magnitude for adsorbed Rhodamine B molecules, in comparison with the mechanically exfoliated graphene [27]. The chemical enhancement from GO has been shown to arise from π - π stacking and charge transfer from the lone-pairs of electrons on the oxygen-containing moieties to the probe molecule [28, 29]. The GO reduction degree may be a key-point for the capacity of the nanocomposites in the detection of Raman probe molecule.

4. Conclusions

This research had focus on the preparation of a freestanding and flexible surface-enhanced Raman scattering (SERS) substrate, where Ag NPs or AgGO nanocomposites were introduced in PAN fibres produced via electrospinning technique.

We tested two different methodologies to coat PAN electrospun network fibres with Ag NPs and AgGO nanocomposites. Both methodologies could be

effective. However a precise control of the Ag nanoparticles content and size, as well as the GO reduction degree and also content, still need to be optimized to increase the SERS efficiency of these substrates.

This work represents a positive contribution for the understanding of the role of GO as an emerging material for SERS detection. Its introduction on easy handled substrates prepared by electrospinning that can be disposable and low-cost produced when compared with other conventional SERS substrates, implying that it is a perfect choice for practical SERS detection application should continue to be explored.

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