

Glassy carbon electrodes modified by multiwalled carbon nanotubes and poly(neutral red): A comparative study of different brands and application to electrocatalytic ascorbate determination

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Abstract The electrochemical behaviour of glassy carbon electrodes coated with multiwalled carbon nanotubes (MWCNT) from three different sources and with different loadings has been compared, with a view to sensor applications. Additionally, poly(neutral red) (PNR) was electro-synthesised by potential cycling on bare glassy carbon and on MWCNT-modified glassy carbon electrodes, and characterised by cyclic voltammetry and scanning electron microscopy. Well-defined voltammetric responses were observed for hexacyanoferrate (II) oxidation with differences between the MWCNT types as well as from loading. The MWCNT and PNR/MWCNT-modified electrodes were applied to the oxidative determination of ascorbate, the electrocatalytic effects observed varying according to the type of nanotubes. Comparison was made with electrodes surface-modified by graphite powder. All modified electrode configurations with and without PNR were successfully employed for ascorbate oxidation at +0.05 V vs saturated calomel electrode with detection limits down to 4 μ M; good operational stability and storage stability were also obtained.

Keywords Multiwalled carbon nanotubes · Poly(neutral red) · Ascorbate · Modified electrodes

Introduction

Since the discovery of carbon nanotubes (CNT) by Iijima [1] in 1991, the prospects for their application have ranged from

reinforced composites to molecular-scale electronic devices. CNTs can be used as electrode materials with useful properties for electrochemical and bioelectrochemical applications, owing to unique characteristics such as high conductivity, high aspect ratio, high chemical stability and extremely high mechanical strength and modulus [2–5]. Usually, CNT are produced by chemical vapour deposition (CVD) or arc discharge processes, and it was observed in previous work [6] that the electrochemical reactivity depends on the production method, with the CVD leading to higher electrocatalytic activity. Whereas single-walled CNTs are comprised of a cylindrical-structure graphene sheet of nanoscale diameter capped by hemispherical ends [7], multiwalled CNTs (MWCNT) are comprised of several to tens of concentric cylinders of these graphene sheets with a layer spacing of 0.3–0.4 nm. MWCNTs have external diameters in the range 2–100 nm and can be considered as a mesoscale graphite system; they can also exist in herringbone and bamboo configurations [8, 9].

The special CNT structure enables them to have more electrochemically active sites (e.g. edge plane-like carbons), as compared with, for example, glassy carbon and graphite, which makes them very attractive for electrochemical determinations at low potentials. CNTs can act as support for organic and inorganic electrocatalysts to form electrochemically functional nanodevices and sensors [10], which can be ascribed to their highly π -conjugated and hydrophobic sidewalls consisting of sp^2 carbons and open ends with oxygen-containing moieties [11]. These unique structural and electronic properties make them able to form 3-D conducting matrices that can be used for the immobilisation of enzymes [12] and DNA [13], allowing direct electron transfer to and from those biomacromolecules with enhanced faradaic responses [14]. For such purposes, it is necessary to

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functionalise the sidewalls of CNTs in non-covalent ways in order to preserve the sp^2 nanotube structure and thus their electronic characteristics [15–17]. Due to the hydrophobic nature of CNT, dispersions of them in aqueous solutions and organic solvents are not easy to prepare. To impart a hydrophilic nature and chemically activate the defect sites at the same time, the MWCNT need to be purified and chemically functionalised. These treatments can be done by chemical methods: for example, oxidation with strong oxidising acids is an effective way to generate $-COOH$ groups on their surface [18].

In recent years, carbon nanotube/conducting polymer composite modified electrodes have been the object of much interest because the incorporation of CNTs into conducting polymers can lead to new composite materials with synergic effects [8]. The π -conjugated structure of the nanotubes allows them to interact with organic aromatic compounds through π - π electronic and hydrophobic interactions [19, 20].

Polyazine electroactive polymers have been shown to have good conducting characteristics as well as preserving the redox behaviour of the monomers, particularly useful for applications in sensors and biosensors [21–23]. The phenazine dye neutral red (NR), like other azines, has often been used as a biological marker, an indicator of pH in biochemical systems, an artificial enzyme substrate and a redox mediator to determine lactate, glutamate, glucose and other important analytes [24, 25]. Since NR has a second heteroatom on the phenazine ring, which is a nitrogen instead of a divalent oxygen or sulphur, it has a much lower redox potential than analogous phenothiazines and phenoxazines [26], and the amino functionality makes it amenable to electropolymerisation. Electropolymerisation to give poly(neutral red) (PNR) with a view to ensuring long-term stability and retention of electrochemical response of the NR redox-active sites has been done on various electrode substrates such as, for example, carbon film [23, 24]. The PNR film, together with CNTs, may lead to a synergic improvement in electrochemical sensor properties, which is investigated in this work.

Ascorbic acid/ascorbate is a vital component in the diet of humans. Ascorbate prevents scurvy and is known to take part in a number of biological reactions. It is arguably the primary antioxidant in human blood plasma and is present in the mammalian brain in the presence of various neurotransmitter amines including dopamine, epinephrine and norepinephrine [27]. Direct and selective detection of ascorbate at conventional or metal electrodes is difficult due to its large overpotential and electrode fouling by oxidation products [28]. Many strategies have been developed, using different types of modified electrode, to reduce the overpotential for the catalytic electrooxidation of ascorbate, as indicated in Ref. [29].

The objective of the present work was to evaluate the differences in electrocatalytic effects between different types of MWCNT, as well as the influence of loading, used in MWCNT-modified and PNR/MWCNT-modified glassy carbon electrodes. This was first done for hexacyanoferrate (II) oxidation, as model electroactive species, and then applied as a sensor, illustrated by the electrocatalytic oxidation of ascorbate.

Experimental

Reagents and solutions

Multiwalled carbon nanotubes were: MWCNT_{Sig} from Sigma, Germany with >90% purity, 110–170 nm diameter and 5–9 μm length; MWCNT_{She} from Shenzhen Nanotech, China with >90% purity, 40–60 nm diameter and 5–15 μm length; and MWCNT_{Nan} from NanoLab, USA, with ~95% purity, 30 ± 10 nm diameter and 1–5 μm length.

Graphite powder (grade no. 38) was obtained from Fisher Scientific, USA.

N,N-Dimethylformamide (DMF), neutral red-65% dye content, potassium hexacyanoferrate (II) trihydrate and potassium nitrate were obtained from Fluka, Switzerland. Potassium hydrogen phosphate, di-sodium hydrogen phosphate 2-hydrate, sodium chloride and nitric acid were from Riedel-de Haën, Germany, and ascorbic acid was from Sigma-Aldrich, Germany. Potassium hydrogen phosphate 3-hydrate was from Panreac, Spain. All reagents were of analytical grade and were used without further purification.

Buffer solutions of 0.025 M potassium phosphate saline buffer (KPBS) pH 5.55 were prepared from potassium hydrogen phosphate and di-potassium hydrogen phosphate 3-hydrate.

Standard 1 mM potassium hexacyanoferrate (II) solutions were prepared by dissolving the salts in 0.1 M potassium chloride electrolyte solution. Ascorbic acid solutions were freshly prepared for each experiment.

The solution used for electropolymerisation of NR contained 1 mM NR in 0.025 M KPBS pH 5.55 buffer solution, as described above, with the addition of 0.1 M potassium nitrate.

Millipore Milli-Q nanopure water (resistivity $\geq 18\text{ M}\Omega\text{ cm}$) was used for the preparation of all solutions. All experiments were performed at room temperature ($25\pm 1\text{ }^\circ\text{C}$).

Apparatus

A three-electrode electrochemical cell of 10 mL volume was used, containing the glassy carbon (GC) (area 0.28 cm^2), MWCNT-modified or PNR/MWCNT-modified GC electrode as working electrode, a platinum foil counter

electrode and a saturated calomel electrode (SCE) as reference. The GC electrode surface was polished using diamond spray (Kemet International, UK) on a polishing cloth down to 1- μm particle size. It was then rinsed and ultrasonicated in Milli-Q ultrapure water.

All electrochemical measurements were performed using a computer-controlled μ -Autolab Type II potentiostat-galvanostat (Metrohm-Autolab, Utrecht, Netherlands) running with GPES (General Purpose Electrochemical System) for Windows version 4.9 software.

Microscope images were acquired on indium tin oxide (ITO) electrodes (ITO-coated glass slides, Sigma-Aldrich) using a scanning electron microscope (SEM) Jeol JSM-5310 (Jeol, Tokyo, Japan), equipped with a thermionic field emission SEM and an electronically controlled automatic gun. All images were captured at 20 kV.

The pH measurements were carried out with a CRISON 2001 micro pH-meter (Crison, Spain) at room temperature.

MWCNT functionalisation, electrode coating and NR polymerisation

A mass of 120 mg of MWCNT was stirred in 10 mL of a 3 M nitric acid solution for 24 h. The solid product was collected on a filter paper and washed several times with nanopure water until the filtrate solution became close to neutral (pH \approx 7). The functionalised MWCNTs obtained were then dried in an oven at 80 $^{\circ}\text{C}$ for 24 h.

The dried MWCNTs were dispersed in DMF, 0.2% and 1% (m/v), and sonicated for 4 h to ensure a homogeneous mixture. The MWCNT coating was prepared by dropping 10 μL of the CNT/DMF dispersion on the surface of the GC electrode using a micropipette and allowing it to dry at room temperature; this procedure was done twice, leading to a film with \sim 0.14 or \sim 0.70 mg cm^{-2} of MWCNT.

A dispersion of 1% (m/v) of graphite powder (GP) in DMF was also made. After sonicating for 1 h, a homogeneous mixture was obtained and used to coat the GC electrode in the same way as for the MWCNT.

PNR phenazine polymer was prepared as a film on the GC and on MWCNT/GC electrodes by potential cycling at a scan rate of 50 mV s^{-1} . The potential was cycled between -1.0 and $+1.0$ V vs SCE for 15 cycles.

Results and discussion

Electrochemical characterisation of MWCNT-coated electrodes

The MWCNT-modified GC electrodes, coated with different types of MWCNT, were characterised by cyclic voltammetry (CV) of a solution of 1.0 mM potassium ferrocyanide in 0.1 M

KCl supporting electrolyte using scan rates from 10 to 100 mV s^{-1} . In the presence of MWCNTs, the capacitance values, C , determined from the capacitive background currents, were significantly higher than those of the bare GC electrode, showing that the modification with nanotubes leads to an increase in the available carbon surface area and increases with nanotube loading, as expected (Table 1). The capacitance values also vary between the different brands of nanotubes, which could be related to the fact that MWCNTs present different amount of defects for each brand of MWCNT; the values from the NanoLab CNT are clearly the largest.

Well-defined redox peaks were obtained for the cyclic voltammetry of hexacyanoferrate (II), with mid-point potential at ca. 0.165 V vs SCE superimposed on the capacitive background current; background-subtracted CVs are shown in Fig. 1. The shapes of the voltammograms were only slightly affected by the scan rate, and both cathodic and anodic peak currents depend linearly on the square root of the scan rate (over the whole range of scan rates examined) as expected, reflecting the fact that the electrochemical process is diffusion-controlled.

Comparing the different brands and loadings of CNTs (see Fig. 1), the higher loading (Fig. 1b) leads to sharper peaks with smaller peak separation (\approx 63 mV), suggesting an increase in electrochemical reversibility. At the same time, the background current also increased (not shown), indicating that the non-electroactive surface area is also enhanced by CNTs.

The electroactive surface area was calculated using the Randles–Sevcik equation

$$I_p = 2.69 \times 10^5 AD^{1/2} n^{3/2} \nu^{1/2} C \quad (1)$$

where I_p is the oxidation peak current (in A), A is the electroactive area (in cm^2), D is the diffusion coefficient of the electroactive species (in $\text{cm}^2 \text{s}^{-1}$), n is the number of electrons transferred, ν is the potential scan rate (in V s^{-1}) and C is the concentration of the redox species in bulk solution (in mol cm^{-3}) [30]. The diffusion coefficient of hexacyanoferrate (II) in 0.1 M KCl solution at the bare GC electrode was calculated using chronoamperometry as described in [30] to be $6.20 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. Table 1 shows the values of the calculated electroactive areas (A_{elc}) and the ratio of electroactive to geometric area. First, it is seen that not all the geometric area of the bare GC electrode is electrochemically active, as what often happens. Secondly, the presence of carbon nanotubes not only leads to an increase of the electroactive area, related to the MWCNT loading, but also depends on the brand of nanotubes. These experiments were carried out three times to test reproducibility and gave the same results.

Table 1 Characteristics of MWCNT/GC electrodes: electroactive area, A_{ele} , related to the geometric area, A_{geo} , capacities, C , and standard electron transfer rate constants, k_0 , from CVs in the scan

MWCNT loading (% m/v)	GC	0.2%			1.0%		
		MWCNT _{Sig}	MWCNT _{Shu}	MWCNT _{Nan}	MWCNT _{Sig}	MWCNT _{Shu}	MWCNT _{Nan}
$A_{\text{ele}}/\text{cm}^2$	0.21 ± 0.004	0.31 ± 0.015	0.31 ± 0.008	0.33 ± 0.007	0.75 ± 0.010	0.72 ± 0.010	0.91 ± 0.010
$(A_{\text{ele}}/A_{\text{geo}})/\%$	75	111	111	118	268	257	325
$C/\text{mF cm}^{-2}$	0.090 ± 0.003	0.81 ± 0.02	0.24 ± 0.01	1.64 ± 0.01	5.62 ± 0.08	1.81 ± 0.01	9.41 ± 0.04
$k_0 \times 10^{-3}/\text{cm s}^{-1}$	2.7	6.7	4.7	7.1	17.9	16.4	19.5
PNR/MWCNT: surface coverage of redox sites/ nmol cm^{-2}	4.9	7.1	6.7	9.7	14	19	22

The difference between oxidation and reduction peak potential values was larger than the ideal value of 57 mV for fast electrode kinetics and increased slightly with increasing scan rate, as is common on carbon electrode surfaces [31]. Using the electroactive area A_{ele} , the apparent standard electron transfer rate constants were also calculated, as described in [30]. Electrodes with a higher loading of

nanotubes appear to present a more reversible behaviour; this could be due partly to the easier access to the most active sites.

Electrochemical characterisation of PNR and PNR/MWCNT-modified electrodes

Previous studies have shown that the protonation of leuco-NR and NR is pH dependent [21]. Varying the pH in phosphate buffer solutions in the range 5.0 to 8.0 (data not shown) demonstrated that electropolymerisation of NR occurs best in phosphate buffer pH 5.55, which was used in all further experiments.

Electropolymerisation begins with adsorption of the monomer and formation of monomer cation radicals at positive potentials, followed by chain growth. From Fig. 2, it is seen that NR electropolymerises more readily at MWCNT-coated than at bare glassy carbon electrodes. The NR/leuco-NR peaks (couple I) are less well-defined with MWCNT, but the

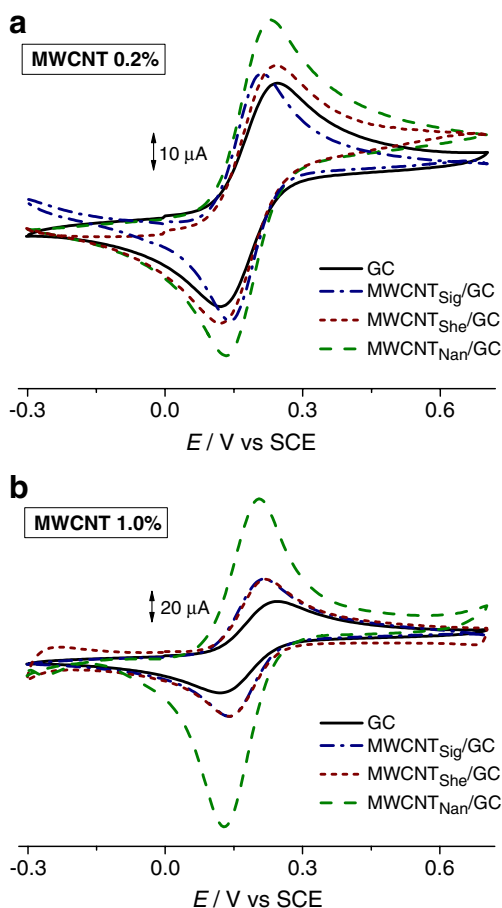


Fig. 1 Background-subtracted cyclic voltammograms at 50 mV s^{-1} at GC electrodes with MWCNT **a** 0.2% (m/v) and **b** 1% (m/v) in 1 mM $\text{K}_4\text{Fe}(\text{CN})_6$ in 0.1 M KCl solution

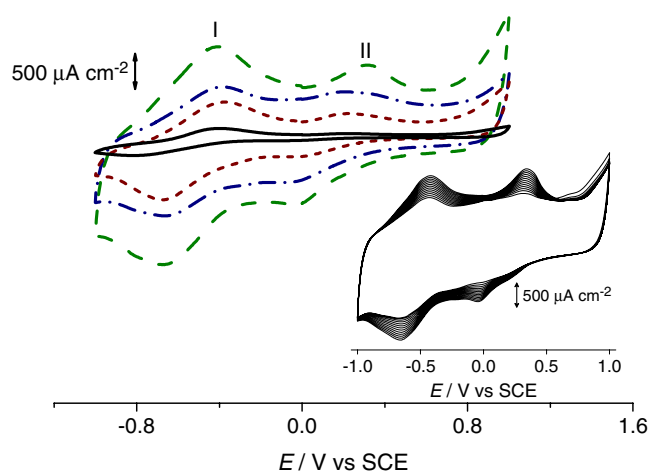


Fig. 2 Cyclic voltammograms of PNR films on GC (black lines) and GC modified with MWCNT from (---) Sigma, (· · ·) Shenzhen and (– · –) Nanolab in KPBS at 50 mV s^{-1} scan rate. Peaks (I) and (II) represent the two redox couples of PNR. Inset shows electropolymerisation of NR on an MWCNT_{Nan} (1%)/GC electrode

redox couple II is much better defined than at bare GC. An additional redox wave appears between these redox couples, probably due to two-step NR oxidation to leuco-NR [32, 33]. The redox reaction of the monomer and oligomer that formed during electropolymerisation occurred at $E^{o'} = -0.41$ V vs SCE, while at $E^{o'} = +0.31$ V vs SCE, the redox reaction of the polymer and oligomer takes place. Moreover, an increase in PNR peak currents was observed for both redox couples I and II with the higher MWCNT loading since the carbon nanotubes may be able to provide an easier electron transfer pathway and enhance the polymerisation process.

Figure 3 compares the influence of the type and loading of the MWCNT, in terms of the relation between the charge capacity of each modified electrode. It is observed that the polymer film increases the charge capacity, which is directly connected to the amount of nanotubes present on the surface of the electrode, as well as to the influence of the MWCNT source, those from NanoLab showing a higher charge capacity ($\approx 25\%$) for all types of modification (different CNT loadings, both with and without PNR).

In order to quantify the influence of the presence of the MWCNT, the PNR redox site surface concentration (Γ) was calculated for each modified electrode. The CVs at 50 mV s^{-1} were used to obtain the charges (Q) involved in each process (I) and (II). The values obtained were substituted in the equation $\Gamma = Q/(nFA)$, where n is the number of electrons, A is the electrode area (cm^2) and F is the Faraday constant. The redox activity of the PNR on GC/MWCNT is an electrochemical process that involves two electrons and two protons per site [34], thus $n=2$. Table 1 gives the values obtained. Regarding the type and loading of nanotubes, the results obtained from cyclic voltammetry are consistent with the relative amounts of PNR present on the different electrodes.

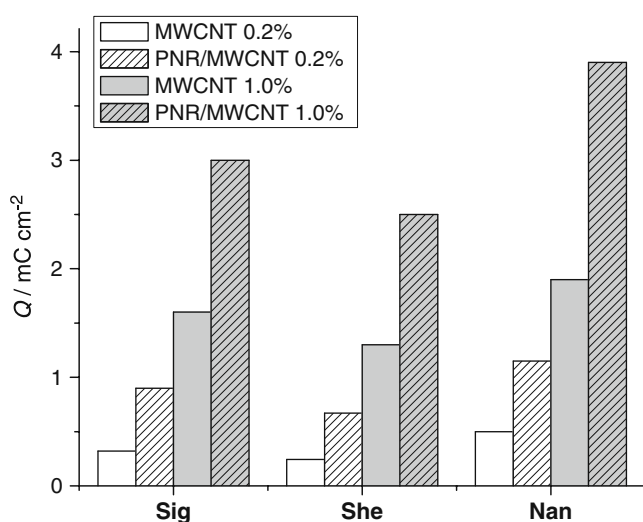


Fig. 3 Values of charge under the polymer redox peaks for the GC electrodes modified with different types and loadings of MWCNT without and with PNR

From Table 1, it is seen that the MWCNT from NanoLab are the most suited for the electropolymerisation of NR, due to the higher Γ values obtained, and that higher loadings of CNT lead to an increase of the amount of PNR, also deduced from the higher peak currents as mentioned above.

In a previous work, it was usually observed that the rate-determining process at PNR was rather complicated because the PNR anodic and cathodic peaks had a different behaviour depending on the charge compensation process in the film. Diffusion through the PNR films was the rate-determining step at ITO [34] or gold [31] at low pH values and also at carbon film electrodes [27]. In this work, at the bare GC electrode and at the modified electrodes with lower loading of MWCNT, the redox process is diffusion-controlled as in the cases mentioned. However, for a higher loading of CNTs, Fig. 4 shows that I_p depends linearly on scan rate, up to 200 mV s^{-1} for both anodic peaks I and II (see inset), indicating a surface-controlled process, and according to several reports [35–37] in the presence of a transition to thin-layer behaviour. Moreover, the linear dependence with scan rate observed for only this modification would also be seen if there was adsorption of species in the surface layer, the distinction between these two processes not being easy to make, although adsorption can be seen as the limit of thin-layer diffusion [38]. In this case where the oxidation of ascorbate was carried out with the GC/MWCNT/PNR (1% m/v) electrode after transferring it to a fresh electrolyte solution without ascorbate, the changes observed in the voltammograms were relatively small (ascribed to memory effect), indicating that thin-layer effects may dominate [36].

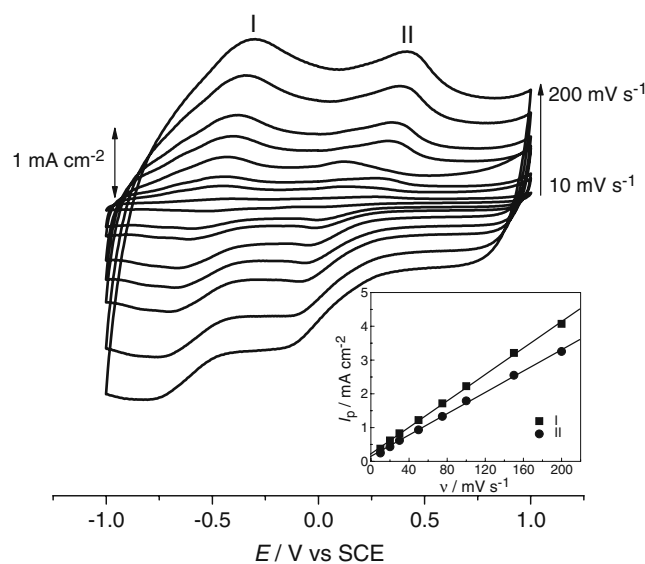


Fig. 4 Cyclic voltammograms of PNR/MWCNT_{Nan} (1%)/GC in KPBS at different scan rates from 10 to 200 mV s^{-1} . The inset is a plot of anodic peak currents (I) and (II) vs scan rate

Scanning electron microscopy

SEM images can provide information on the electrode surface morphology. For this purpose, PNR, $\text{MWCNT}_{\text{Nan}}$ and $\text{MWCNT}_{\text{Nan}}/\text{PNR}$ composite films were prepared on ITO substrates under similar conditions to those used to prepare the same films on GC (cyclic voltammetry experiments gave the same behaviour as on GC substrates). Figure 5a shows

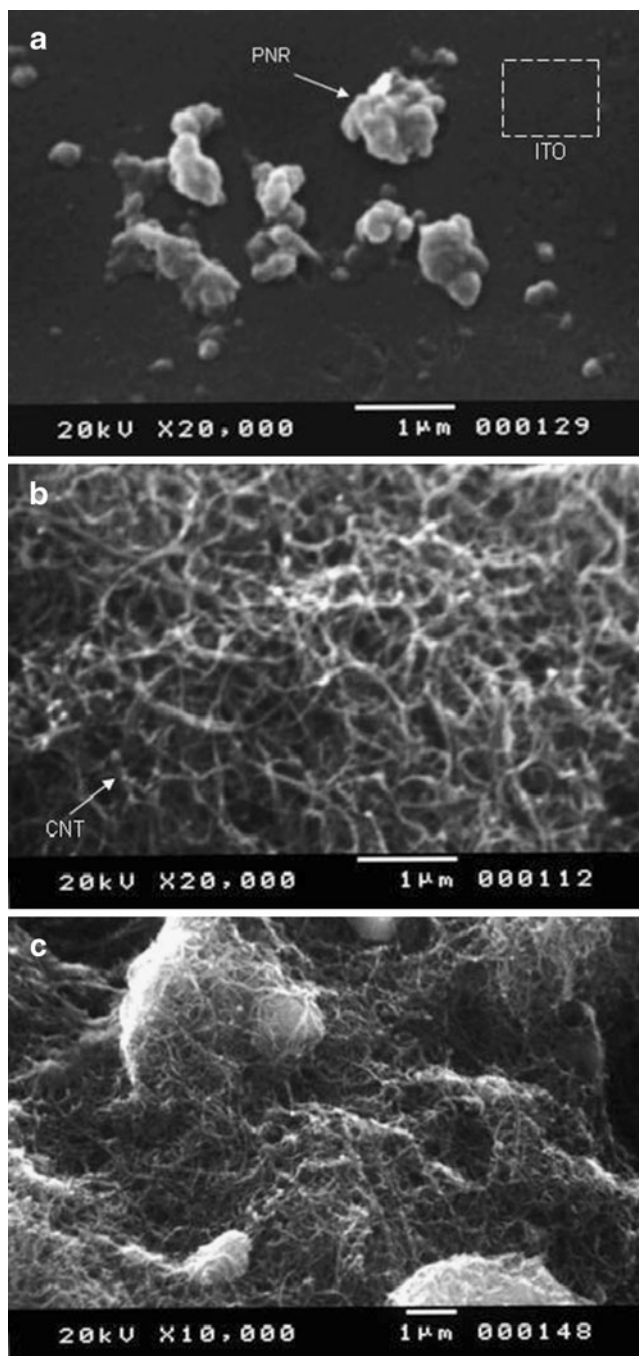


Fig. 5 Scanning electron micrographs on ITO substrate electrodes of **a** PNR, **b** $\text{MWCNT}_{\text{Nan}}$ (1%) and **c** $\text{PNR}/\text{MWCNT}_{\text{Nan}}$ (1%)

PNR on the ITO electrode surface, which reveals that PNR by itself forms separate agglomerates; the bare ITO surface is shown in the dotted square box.

In Fig. 5b, the presence of MWCNTs is easily seen—multiwalled carbon nanotubes form bundles when large quantities are abrasively immobilised on an electrode surface [31]. The increase in electroactive area compared with Fig. 5a is easily deduced. Due to charging, the lateral dimensions of the MWCNTs in these SEM images will always appear significantly larger than they actually are, resulting in a false impression of the surface coverage. The image in Fig. 5c is in agreement with the observations in the previous sections, i.e. that the amount of PNR on the surface increases when prepared on top of the MWCNT-modified electrode.

Electrochemical oxidation of ascorbate

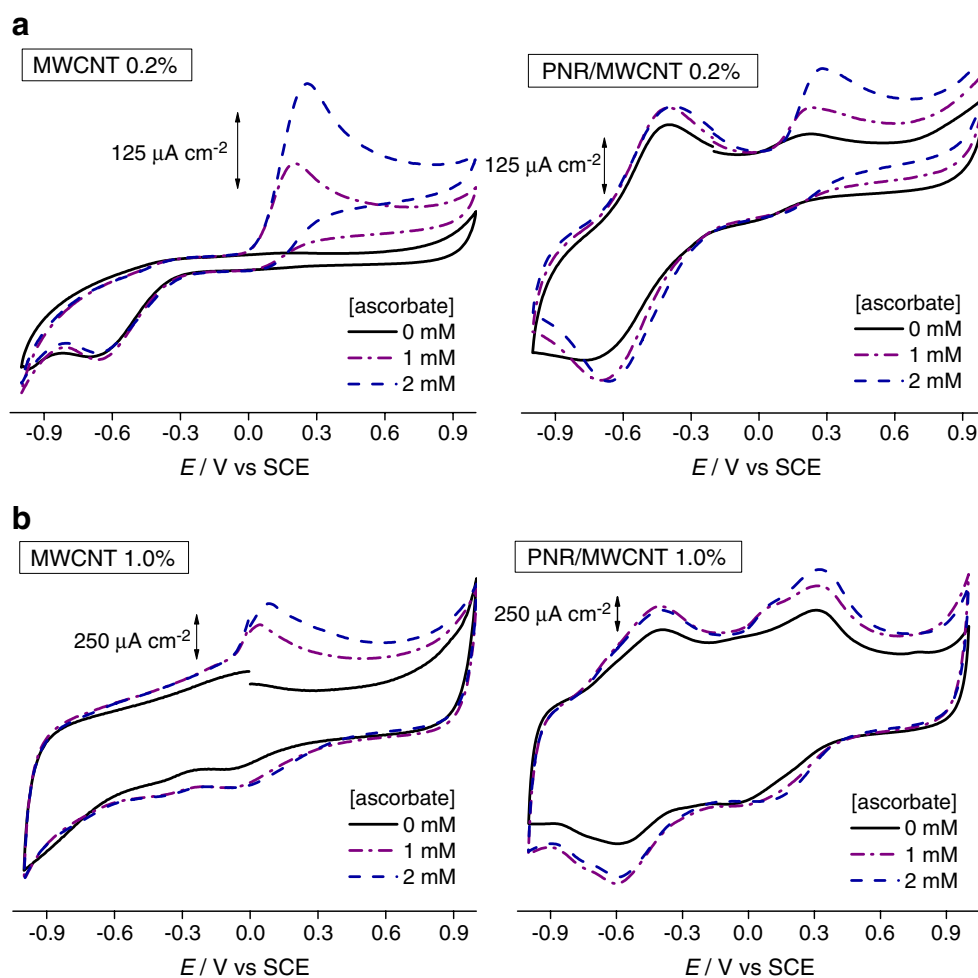
The application of these GC-modified electrodes as sensors for ascorbate by electrocatalytic oxidation was examined in KPBS at pH 5.55.

Cyclic voltammetry

Typical examples of cyclic voltammograms are shown in Fig. 6. As seen from Fig. 6a, the oxidation of 1 mM of ascorbate at the $\text{MWCNT}_{\text{She}}$ (0.2%)/GC electrode occurred irreversibly at +0.20 V vs SCE, whilst with a higher loading, of 1%, oxidation occurred at a lower potential of +0.08 V vs SCE (Fig. 6c). At bare GC under the same conditions, the ascorbate oxidation peak appears at +0.4 V vs SCE (data not shown). Therefore, MWCNT have an electrocatalytic effect on the oxidation of ascorbate promoted by oxygen-containing functionalities, as also observed in [39]. Table 2 shows the peak potentials for all brands of nanotubes for 1% loading, where the greatest electrocatalytic effect is with $\text{MWCNT}_{\text{Nan}}/\text{GC}$ at +0.07 V vs SCE. With an increase of nanotube loading, an increase in the background current and in the ascorbate oxidation peak current occurred, of 70% for $\text{MWCNT}_{\text{Nan}}/\text{GC}$. The benefits of decreased overpotential and increased oxidation peak current are both seen, providing higher sensitivity and selectivity compared with the bare GC electrode, even with the lower nanotube loadings.

The redox peak currents of the electrodes modified with PNR to ascorbate are also reported in Table 2. Cyclic voltammograms in Fig. 6a, b show the $\text{MWCNT}_{\text{She}}/\text{PNR}/\text{GC}$ electrode response to ascorbate. Unlike without PNR, ascorbate is oxidised at almost the same potential as that of the oligomer and polymer, resulting in an overlapping voltammetric response. Thus, although electrodes modified with PNR respond to different concentrations of ascorbate, the increase of the oxidation peak current is due not only to the presence of the analyte but also to polymer reorganisation.

Fig. 6 Cyclic voltammograms of MWCNT_{She} and PNR/MWCNT_{She} coated GC electrodes in the presence of different concentrations of ascorbate in KPBS pH 5.55. Scan rate 50 mV s⁻¹. **a** MWCNT_{She} (0.2% m/v) and **b** MWCNT_{She} (1.0 m/v)



Furthermore, electrodes modified with 1% (m/v) carbon nanotubes from Sigma and NanoLab had different behaviours to those from Shenzhen. A split in the peaks for the PNR and ascorbate oxidations is observed with the ascorbate oxidation occurring at lower potentials (Table 2). This is extremely

Table 2 Peak potential and peak current for 2 mM ascorbate from cyclic voltammetry at MWCNT/GC and PNR/MWCNT/GC electrodes

Electrodes	0.2% CNT		1.0% CNT	
	E_p/V	$I_p/\mu A$	E_p/V	$I_p/\mu A$
MWCNT _{Sig} /GC	0.229	315	0.047	1,063
MWCNT _{She} /GC	0.257	298	0.033	719
MWCNT _{Nan} /GC	0.225	420	0.071	1,116
PNR/MWCNT _{Sig} /GC	0.271	356	0.129 ^a 0.324 ^b	1,298 ^a
PNR/MWCNT _{She} /GC	0.282	286	0.328 ^b	1,126
PNR/MWCNT _{Nan} /GC	0.251	480	0.129 ^a 0.332 ^b	1,404 ^a

^a Ascorbate oxidation peak

^b PNR oxidation peak in the presence of ascorbate

important for analytical applications, and so the source of the nanotubes used for the PNR/GC surface modification can increase and separate oxidation responses, owing to the enhanced electrocatalytic activity [40].

Some authors advise caution in assigning any unique electrocatalytic properties to CNTs alone [39]. When CNTs are purified by solubilisation or are chemically treated, it is also important to consider any possible effects of the chemical functionalisation procedure on the CNTs. The solubilisation methods can result in coating of the CNT surface, or attack the CNT structure (in the case of sonication), and these factors can affect the performance. Therefore, understanding the influence of these treatments is clearly important when assessing the electrochemical properties of CNTs [39] and may affect CNTs made by different preparation procedures in different ways.

Fixed potential amperometry

The electroanalytical determination of ascorbate by fixed potential amperometry was carried out at 0.0, +0.05 and +0.1 V vs SCE in order to optimise the applied potential value

(data not shown). At 0.0 V vs SCE, only the electrodes modified with higher loadings of MWCNT responded, and so +0.05 V vs SCE was chosen for further experiments since at this potential all electrode assemblies had a sufficiently good response to enable ascorbate determination in the micromolar range (Fig. 7).

Analytical parameters calculated from calibration curves at +0.05 V vs SCE are given in Table 3. As expected, at +0.05 V vs SCE, the sensitivity was lowest for the unmodified glassy carbon electrode ($1.2 \pm 0.07 \mu\text{A mM}^{-1} \text{cm}^{-2}$), and the detection limit was highest at $13.9 \mu\text{M}$. At the electrodes covered with the MWCNT 0.2% (m/v), an increase of 100 times in sensitivity to ascorbate was found compared with bare glassy carbon. With the higher loading of MWCNT, the increase in sensitivity was greater, with values around $300 \mu\text{A mM}^{-1} \text{cm}^{-2}$, attributable to the increase of the electroactive area. Modification with MWCNT_{Nan} led to the

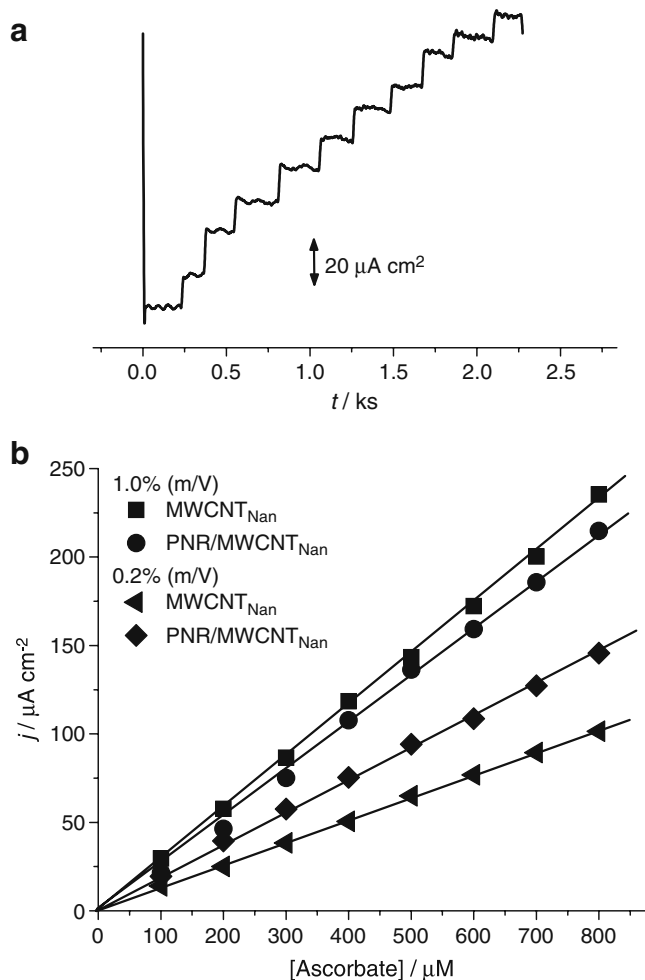


Fig. 7 Determination of ascorbate by fixed potential amperometry at +0.05 V vs SCE at MWCNT_{Nan} and PNR/MWCNT_{Nan}-modified electrodes. **a** Typical amperometric response for PNR/MWCNT_{Nan}/GC, for successive additions of 50 μL of 0.1 M of ascorbate ($\sim 100 \mu\text{M}$) in KPBS pH 5.55. **b** Calibration plots at various modified electrodes

Table 3 Analytical parameters from ascorbate calibration curves recorded at GC and at MWCNT and PNR/MWCNT-modified GC electrodes at +0.05 V vs SCE, solution KPBS pH 5.55

MWCNT loading (% m/v)	0.2%				1%					
	GC	PNR/GC	MWCNT _{Sig}	MWCNT _{She}	MWCNT _{Nan}	PNR/MWCNT _{She}	MWCNT _{Sig}	PNR/MWCNT _{Nan}	PNR/MWCNT _{She}	PNR/MWCNT _{Nan}
Sensitivity ($\mu\text{A cm}^{-2} \text{mM}^{-1}$)	1.2 ± 0.1	4.3 ± 0.3	169.4 ± 1.1	201.5 ± 0.7	200.2 ± 1.2	187.6 ± 0.9	357.8 ± 0.6	334.9 ± 0.5	383.7 ± 0.6	375.2 ± 0.8
LOD (μM)	13.9 ± 0.2	12.1 ± 0.3	8.5 ± 0.1	7.6 ± 0.3	8.8 ± 0.1	8.7 ± 0.2	5.3 ± 0.2	5.9 ± 0.1	4.3 ± 0.1	4.7 ± 0.3
Correlation coefficient (R^2)	0.9987	0.9989	0.9992	0.9996	0.9992	0.9992	0.9985	0.9991	0.9991	0.9993

best electrochemical response, with higher sensitivity and lower detection limits, even without PNR redox mediator, down to 4 μM . This detection limit is almost as low as the 3 μM found at graphite composite electrodes in [29] and is lower than other ascorbate sensors described in the literature at these applied potentials (see [29] for a summary). A linear response ($R^2 \approx 0.999$) to ascorbate was obtained up to 800 μM , equal for all modified electrodes, compared with 80 μM at GC and 140 μM at PNR/GC electrodes.

Regarding the influence of PNR, an increase of 15% in sensitivity was observed for the PNR/MWCNT (0.2%) modified GC electrodes (Table 3) compared with without PNR. However, for the higher loading of 1% MWCNT, the presence of the polymer film does not provide any further increase in sensitivity towards ascorbate oxidation (Fig. 7), unlike what was found by cyclic voltammetry.

Stability of MWCNT/GC and PNR/MWCNT/GC electrodes

The stability of the electrodes is also an important factor. The oxidation peak current of 2 mM ascorbate was measured after 200 cycles at 50 mV s^{-1} in KPBS pH 5.55 and also after the electrodes were immersed in buffer electrolyte for 3 days and for 4 weeks. All types of MWCNT-modified electrode presented the same behaviour with respect to any loss in response. The results are collected in Table 4.

It can be seen that the loss in response is small after 200 cycles. After 3 days' storage, there is a small decrease with MWCNT alone and a small increase with PNR present, probably due to some conformational changes on the electrode surface. After 4 weeks, there was a significant decrease in the electrochemical response, the biggest decrease (46%) being for MWCNT (1%) modified GC. This behaviour can be explained to an ineffective adhesion of the nanotubes to the electrode surface observed when working with higher loadings. This was not observed at electrodes modified with PNR or electrodes with a lower loading of nanotubes. Thus, although the PNR film does not increase the sensitivity of the response of electrodes modified with 1% (m/v) of MWCNT, the film stabilises the structure of the electrode surface since it acts as an adherent allowing for better adhesion of the carbon nanotubes, which

will be of greater importance for high concentrations of CNT.

Assessment of CNT structure and purity on sensor behaviour

Differences in the electrochemical activity between the CNT-modified electrodes and the bare glassy carbon electrodes can be attributed to the surface chemistry (primarily to defects) of the CNTs, from the different brands, although the same CVD production methodology was used in all three MWCNTs tested. Previous authors [41, 42] have stated that the differences in electrocatalytic activities of electrodes modified with CNTs were dependent on the CNT fabrication method. For example, there is a dramatic difference in the effect of pretreatment on arc- and CVD-produced CNT on the electrochemical behaviour of model redox systems such as ascorbic acid, NADH and hydrazine. In this work, the nanotubes used were all produced by the CVD method in order to minimise the structural variables.

Amorphous carbon is the more reactive form of carbon present in as-made CNTs [43], and therefore, the amount of amorphous carbon must be taken into consideration on the catalytic behaviour of CNTs. Using graphite powder (GP) as the electrocatalyst instead of nanotubes, Compton et al. [44] found similar catalytic behaviour.

Thus, the cyclic voltammetric response of GC electrodes modified with GP was explored using a 1 mM aqueous solution of potassium ferrocyanide in 0.1 M KCl, in the scan range 0.01–0.15 V s^{-1} . The results obtained suggested a reversible behaviour similar to that obtained for the modifications with the nanotubes, where the peak-to-peak separation was 93 mV at 50 mV s^{-1} with mid-point potential at ca. 0.185 V vs SCE and an increase of peak separation with increasing scan rate (data not shown). Comparison of the results obtained with unmodified GC and GC modified with MWCNT or GP suggests that the graphite powder has a similar response as an electrocatalytic material as carbon nanotubes, as seen in [44].

Figure 8 compares cyclic voltammograms obtained with GC, GC/GP and GC/MWCNT for 1 mM ascorbate in 0.1 M KPBS pH 5.5 at 50 mV s^{-1} scan rate. For the GC/GP electrode, an oxidation peak was observed at around +0.01 V vs SCE. This value is almost identical to the peak potential of ascorbate observed with the GC modified with MWCNT. Comparison of the response obtained for the GC/GP and GC/MWCNT-modified electrodes suggests that the latter has a more sensitive electrochemical response towards ascorbate oxidation, but with the former also presenting electrocatalytic behaviour.

Although these results suggest that the presence of amorphous carbon in MWCNT may lead to an increase of electrocatalytic behaviour of the nanotubes, the work of Shao

Table 4 Variation of the oxidation peak current response (%) of the modified electrodes, measured by cyclic voltammetry at 50 mV s^{-1} , for addition of 2 mM ascorbate

Electrode modifier	200 scans	3 days	4 weeks
MWCNT 0.2%	-4	-1	-32
PNR/MWCNT 0.2%	-5	+2	-27
MWCNT 1.0%	-2	-5	-46
PNR/MWCNT 1.0%	-2.5	+3	-25

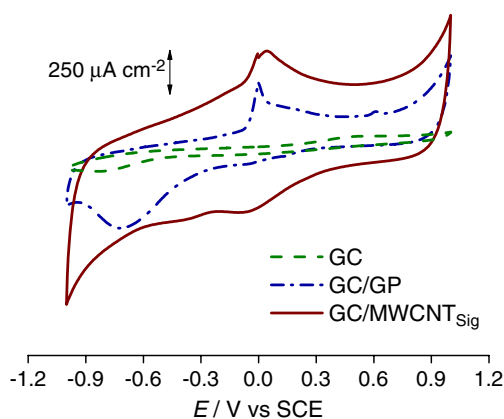


Fig. 8 Cyclic voltammograms of GC, GC/GP and GC/MWCNT_{Sig} (1.0% *m/v*) electrodes in the presence of 1 mM ascorbate in KPBS pH 5.55. Scan rate 50 mV s⁻¹

et al. [43] shows that sidewall functionalisation of carbon nanotubes using the standard nitric acid treatment can be greatly enhanced by first removing the amorphous carbon present in the sample. In this chemical treatment, oxidation debris (carboxylated carbonaceous fragments (CCFs)) are formed due to the functionalisation of the amorphous carbon. The CCFs then create a continuous coating on the carbon nanotube walls, preventing attack on the carbon nanotubes themselves.

In spite of the fact that the electrocatalytic behaviour cannot be due not only to the carbon nanotubes but also to the amorphous carbon present in them (Fig. 8), its removal should be considered before doing any chemical treatment, as it can dominate the features of bulk measurements done on carbon nanotube samples, preventing the sidewall functionalisation of the CNTs [43].

It has been reported that the origin of the heterogeneous charge transfer kinetics for CNTs are exclusively due to the edge plane-like sites defects, but more recent work demonstrated that metallic-based impurities were also responsible for the redox reactions at acid purified CNTs containing residual nanoparticles (NPs) [45–49]. X-ray photoelectron spectroscopy demonstrated that the unwashed MWCNT studied contain 1.0 at.% oxygen, 0.1 at.% iron and trace (<0.1 at.%) copper and sulphur and that the presence of these metal impurities is unaffected by acid washing [45] because the metal nanoparticles are probably trapped in several layers of graphite or contained within the carbon nanotubes [42]. The metallic components such as iron or even copper, despite existing in very small concentrations, were found to participate in electrochemical oxidation processes using MWCNT, e.g. hydrazine [47] or halothane [46], and demonstrated that only a few percent of catalyst nanoparticles are needed to dominate the electrochemical response. Therefore, these contributions to the electrochemical response of CNTs cannot be ignored, and although there are several

methods that can be applied to determine the metallic impurities in CNT [50], most of them can incorrectly give “impurity-free” responses [49]. Also, it is not just the amount of the impurities that is important but also where they are positioned in the nanotube.

In our work, it was observed that the best electrochemical response (PNR polymerization and ascorbate determination) was obtained using CNTs with the highest purity (~95%), but in order to evaluate whether the metallic NPs dominate the electrochemical response, the use of control experiments with carbon nanotubes produced without a metal catalyst [51] would be useful in order to obtain an absolute assessment of the relative contributions of NPs.

According to a previous work, the electroactive surface area of the nanotubes is inversely proportional to their diameter. For example, Zhan’s investigations by transmission electron microscopy and nitrogen adsorption [52] showed that the BET-specific surface area of CNT electrodes decreased with increasing nanotube diameter. The CNT from NanoLab, with the smallest diameter (30±10 nm), had the largest electroactive area per mass concentration and the highest value of the standard electron transfer rate constant for hexacyanoferrate (II) oxidation, as well as leading to a higher PNR surface concentration (see Table 1) and best characteristics for use as a sensor for ascorbate.

Although the lengths of the nanotubes as supplied are different, 1–5 μm (NanoLab), 5–9 μm (Sigma) and 5–15 μm (Shenzhen), this probably had little relevance towards the electrochemical response of the modified electrodes compared with the diameter, for a given mass loading, since the major part of the active sites is on the outer cylindrical surface rather than at the ends of the individual nanotubes. Functionalisation and protonation in strong acid media (in this work HNO₃ was used) and sonication is successful for promoting CNT activity, but often leads to the cutting of the CNTs into smaller pieces, thus losing some of the high aspect ratio [8] and reduces the differences in the length of each brand of nanotubes. The studies undertaken all suggest that the CNT from NanoLab give a superior response in terms of electrochemical behaviour and electrocatalytic response.

Conclusions

The electrocatalytic properties of functionalised multiwall carbon nanotubes from different sources deposited on glassy carbon electrodes have been compared and the effect of electrodeposited poly(neutral red) evaluated. The MWCNT-modified electrodes give well-defined voltammetric responses to the model electroactive species hexacyanoferrate (II), and it was shown that the electroactive area, as well as the standard rate constant for electron transfer, depends on the source and loading of the nanotubes. SEM of MWCNT and PNR on ITO

electrodes confirmed morphological differences between PNR and PNR/MWCNT and that the presence of MWCNT increases the surface concentration of PNR redox sites.

MWCNT/GC and PNR/MWCNT/GC electrodes had excellent functional properties and good electrocatalytic activity towards ascorbate, with detection limits down to 4 μM at +0.05 V vs SCE. Comparison was made with immobilised graphite carbon where electrocatalytic effects were also seen. The electrodes showed good sensitivity for ascorbate determination, depending on nanotube loading and source. Of the three brands tested, MWCNT from Nanolab exhibit the best analytical properties. PNR provides some enhancement in electroanalytical response for the lower MWCNT loadings and in all cases stabilises the modified electrode structure, providing an efficient conducting path through to the electrode substrate. In redox-mediated biosensor applications to be explored in the future, synergic effects can also be expected.

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