

Food Analysis on Microfluidic Devices Using Ultrasensitive Carbon Nanotubes Detectors

A. González Crevillén,[†] Mónica Ávila,[†] Martin Pumera,[‡] María Cristina González,[†] and Alberto Escarpa^{*,†}

Department of Analytical Chemistry and Chemical Engineering, University of Alcalá, E-28871 Alcalá de Henares, Madrid, Spain, and ICYS, National Institute for Materials Science, 1-1 Namiki, Tsukuba, Ibaraki, Japan

Microfluidic devices using carbon nanotube (CNT) materials (single-walled and two multiwalled (MWCNT)) for the analysis of selected analyte groups of significance in foods such as dietary antioxidants, water-soluble vitamins, vanilla flavors, and isoflavones involved in representative food samples have been explored for the first time. Ultrafast separations resulted in well-defined and resolved peaks with enhanced voltammetric current in comparison with those obtained from unmodified screen-printed electrodes, turning MWCNT into an ideal material for electrochemical sensing in food analysis. Resolution was improved by a factor of 2, and sensitivity was dramatically enhanced with amplification factors toward calibration slopes from 4- to 16-fold. In both qualitative and quantitative domains, this impressive performance of CNTs integrated on microfluidics allowed solving specific challenges in food environments such as the direct detection of analytes in complex natural samples and unambiguous analytes in the control of fraud, which was not possible on nonmodified surfaces, avoiding the integration of complex preconcentration steps on these microdevices. The use of these unique materials in microfluidics for food analysis has opened new expectations in “lab-on-a-chip” domains.

It has been demonstrated that microfluidic devices are very attractive analytical microsystems because of their inherent features regarding analysis speed and required low sample volumes.¹ However, lab-on-a-chip technology is not implemented in all research areas with the same degree of intensity. In comparison with clinical applications, food applications constitute a representative example of emerging applications using microfluidic devices that have been recently revised, giving a critical view of its state of the art.² One of the most important differences between clinical and food analysis is how the selectivity and sensitivity are reached. In the clinical field, selectivity and sensitivity are often achieved by using biological materials such as enzymes and antibodies, while in food analysis, this strategy

is less developed, with sample preparation protocols being needed. Food analysis on microfluidics is still in its infancy, and it is now emerging since food samples present complex matrixes where the selectivity and sensitivity become the main challenges. Consequently, in food-related works, CE microchips are used as microsystems to perform fast separations of analytes of high food significance coupled to suitable detectors.^{3–9} Several challenges such as direct detection of analytes in the samples without complex preconcentration steps in authenticity control or fraud detection remain because of the low concentration of these analytes in connection with the extremely low sample volumes introduced in these microdevices. In addition, quantitative data are also very important in food analysis since very strict normative and quality control is based on the accurate measurement of an analyte or cutoff concentration. In consequence, the validation of the methods toward accuracy and precision (reliability) using microfluidic devices is an additional challenge when incorporating these devices in our laboratories. The inherent low concentrations of several food analytes require the systematic use of two different approaches to solve the analysis: to carry out a preconcentration step before separation (which is still performed off-chip because the total integration of analytical steps into microchip format is very difficult¹⁰) or to use ultrasensitive detectors. In the second approach, LIF is the standard detection procedure in microchips¹¹ due to their inherent sensitivity; however, it often needs derivatization schemes. Electrochemical detection (ED) is the most important alternative as a detection route in these microsystems, due to its inherent features of miniaturization without loss of performance and its compatibility with advanced micromachining.¹² Also, many analytes of food significance exhibit more

(3) Scampicchio, M.; Wang, J.; Mannino, S.; Chatrathi, M. P. *J. Chromatogr., A* **2004**, *1049*, 189–194.

(4) Hompesch, R. W.; García, C. D.; Weiss, D. J.; Vivanco, J. M.; Henry, C. S. *Analyst* **2005**, *5*, 694–700.

(5) Blasco, A. J.; Barrigas, I.; González, M. C.; Escarpa, A.; *Electrophoresis* **2005**, *26*, 4664–4673.

(6) Crevillén, A. G.; Blasco, A. J.; González, M. C.; Escarpa, A.; *Electrophoresis* **2006**, *27*, 5110–5118.

(7) Kuban, P.; Hauser, P. C. *Electrophoresis* **2005**, *26*, 3169–3178.

(8) Kato, M.; Gyoten, Y.; Sakai-Kato, K.; Toyo'oka, T. *J. Chromatogr., A* **2003**, *1013*, 183–189.

(9) Blasco, A. J.; Crevillén, A. G.; de la Fuente, P.; González, M. C.; Escarpa, A. *Analyst* **2007**, *132*, 323–329.

(10) Rios, A.; Escarpa, A.; González, M. C.; Crevillén, A. G. *Trends Anal. Chem.* **2006**, *25*, 467–479.

(11) Schwarz, M. A.; Hauser, P. C. *Lab Chip* **2001**, *1*, 1–6.

(12) Wang, J. *Electroanalysis* **2005**, *17*, 1133–1140.

* To whom correspondence should be addressed. E-mail: alberto.escarpa@uah.es.

[†] University of Alcalá.

[‡] National Institute for Materials Science.

(1) Dittrich, P. S.; Tachikawa, K.; Manz, A. *Anal. Chem.* **2006**, *78*, 3887–3908.

(2) Escarpa, A.; González, M. C.; Crevillén, A. G.; Blasco, A. J. *Electrophoresis* **2007**, *28*, 1002–1011.

significant electroactivity than native fluorescence, so only one contribution using LIF was reported in relation to microchip food analysis.⁸ High sensitivity and responses not dependent on optical path length or sample turbidity are additionally very attractive features.

On the other hand, carbon nanotubes (CNTs) are a new group of nanomaterials with unique geometrical, mechanical, electronic, and chemical properties.^{13,14} There are two main types of carbon nanotubes that can have high structural perfection: single-walled nanotube (SWCNT), which consists of a single graphite sheet seamlessly wrapped into a cylindrical tube, and multiwalled nanotube (MWCNT) which comprises an array of such nanotubes that are concentrically nested like rings of tree trunk.^{13,15–18} Since their discovery, CNTs^{15–18} have been widely exploited in electroanalysis because of a common current fad among electroanalysts seeking new surface properties to properly modify a suitable electrode substrate, generally glassy carbon, with a film or layer of CNT. The interesting properties of CNT in electrochemical measurements are the large active surface at electrodes of small dimensions, the enhancement of electronic transfer, and the strong sorption capacity. Therefore, large number of applications can be found in the literature, and several reviews have recently been devoted to this scope.^{19–21}

CNTs have been widely used in electroanalysis; however, these materials have been scarcely explored as detectors in CE microchip and they have never been applied to the real sample analysis. These unique materials were used as microchip detectors for the first time by Wang's group,²² using several analyte classes (carbohydrates, hydrazine, phenol, purine, amino acids, thiols²³) and also exploring the CNT–copper composites in the detection of carbohydrates.²⁴ Recently, Pumera et al. have explored the SWCNT–gold electrodes for microfluidics in the detection of aminophenols and neurotransmitters,²⁵ and the same authors have developed a fundamental study on modification of different electrode materials such as glassy carbon, gold, and platinum surfaces with CNT on a microchip platform using neurotransmitters as model analytes.²⁶ Chen's group used a PMMA/CNT composite electrode for detection of model phenolic pollutants and purines.²⁷ Very recently, Vlčková and Schwartz used CNT-modified electrodes for microchip CE/electrochemistry detection neurotransmitters²⁸ in human urine.

However, the exploration of these novel materials in electrochemical sensing on a CE microchip platform was performed using only standards, and their application in the analysis of real samples (from a broad point of view) and in food analysis (from a particular point of view) is clearly negligible (in both qualitative and quantitative domains). In consequence, new advances from the coupling between the inherent expected benefits from both CE microchips (ultrafast separations, high peak capacity) and CNT-modified electrodes (high sensitivity) in food sample analysis are highly expected.

The purpose of this paper has been to explore the analytical possibilities of CNT coupled to CE microchips as a novel material in the electrochemical sensing of analytes with significance in foods using representative solid samples. To reach this objective, three different CNT materials (SWCNT, MWCNT-A, MWCNT-B) and four groups of analytes of food significance (polyphenols, water-soluble vitamins, vanilla flavors, total isoflavones) covering dietary antioxidants, functional foods, and flavor enhancers were chosen. This food selection was also made because of the need for highly sensitive analysis in the areas of food or nutrient control (evaluation of antioxidant content and reliable detection of frauds and checking quality control in functional foods) in which a relevant role from CNT materials is expected.

EXPERIMENTAL SECTION

Materials and Standards. Folic acid, pyridoxine hydrochloride, genistein, arbutin, phloridzin, catechin, rutin, vanillin alcohol, maltol, ethyl maltol, and ethyl vanillin were purchased from Sigma Chemical Co. (St. Louis, MO). Ascorbic acid and vanillin were from Fluka Chemie (Buchs, UK).

Pharmaceutical preparations (Becozyme C Forte), apples, and natural vanilla beans were purchased in local markets (Madrid, Spain). A secondary standard reference and well-documented in the Drug Master File (SW/1211/03) was used as reference material (confidential, property of Exxentia, Madrid, Spain) for the analysis of isoflavones.

Carbon nanotube materials were obtained from Sigma Aldrich. SWCNT (product no. 519308; length, 2–5 μm ; diameter, 1.2–1.5 nm), MWCNT-200 μm (MWCNT-A; product no. 636630; length, 0.5–200 μm ; o.d., 30–50 nm, wall thickness, 12–18 nm, CVD method produced), and multiwall carbon nanotubes MWCNT-2 μm (MWCNT-B; product no. 636495; 0.5–2 μm ; o.d., 20–30 nm; wall thickness, 1–2 nm, CVD method produced) were purchased from Aldrich. Further purification of carbon material was accomplished by stirring the CNT in 2 M nitric acid (Panreac, Barcelona, Spain) at 80 °C for 24 h and washing with distilled water to neutral pH.²⁹ A 1-mg sample of carbon material was dispersed in 1 mL of dimethyl formamide (DMF, from Sigma-Aldrich) and consequently used for electrode modification.

All chemicals used in buffer preparation and in supporting electrolytes were reagent grade. Methanol and ethanol absolute were acquired from Panreac. In all cases, high-quality water, purified in a Milli-Q system (Millipore, Bedford, MA), was used.

Apparatus and Electrodes. A CE-ED microsystem previously reported³⁰ and recently implemented in our laboratory was used.⁵ The glass chip was fabricated by Micralyne (model MC-BF4-001,

(13) Ajayan, P. M. *Chem. Rev.* **1999**, *99*, 1787–1799.

(14) Trojanowicz, M. *Trends Anal. Chem.* **2006**, *25*, 480–489.

(15) Iijima, S. *Nature* **1991**, *354*, 56–58.

(16) Oberlin, A.; Endo, M.; Koyama, T. *J. Cryst. Growth* **1976**, *32*, 335–349.

(17) Iijima, S.; Ichihashi, T. *Nature* **1993**, *363*, 603–605.

(18) Bethune, D. S.; Kiang, C. H.; De Vries, M. S.; Gorman, G.; Savoy, R.; Vazquez, J.; Beyers, R. *Nature* **1993**, *363*, 605–607.

(19) Wang, J. *Analyst* **2005**, *130*, 421–426.

(20) Merkoçi, A.; Pumera, M.; Llopis, X.; Pérez, B.; del Valle, M.; Alegret, S. *Trends Anal. Chem.* **2005**, *24*, 826–838.

(21) Banks, C. E.; Compton, R. G. *Analyst* **2006**, *131*, 15–21.

(22) Wang, J.; Chen, G.; Chatrathi, M. P.; Musameh, M. *Anal. Chem.* **2004**, *76*, 298–302.

(23) Chen, G.; Zhang, L.; Wang, J. *Talanta* **2004**, *64*, 1018–1023.

(24) Wang, J.; Chen, G.; Wang, M.; Chatrathi, M. P. *Analyst* **2004**, *129*, 512–515.

(25) Pumera, M.; Llopis, X.; Merkoçi, A.; Alegret, S. *Microchim. Acta* **2006**, *152*, 261–265.

(26) Pumera, M.; Merkoçi, A.; Alegret, S. *Electrophoresis* **2007**, *28*, 1274–1280.

(27) Yao, X.; Wu, H.; Wang, J.; Qu, S.; Chen, G. *Chem. Eur. J.* **2007**, *13*, 846–853.

(28) Vlčková, M.; Schwartz, M. A. *J. Chromatogr., A* **2007**, *1142*, 214–221.

(29) Pumera, M.; Merkoçi, A.; Alegret, S. *Sens. Actuators B* **2006**, *113*, 617–622.

(30) Wang, J.; Tian, B.; Sahlin, E. *Anal. Chem.* **1999**, *71*, 5436–5440.

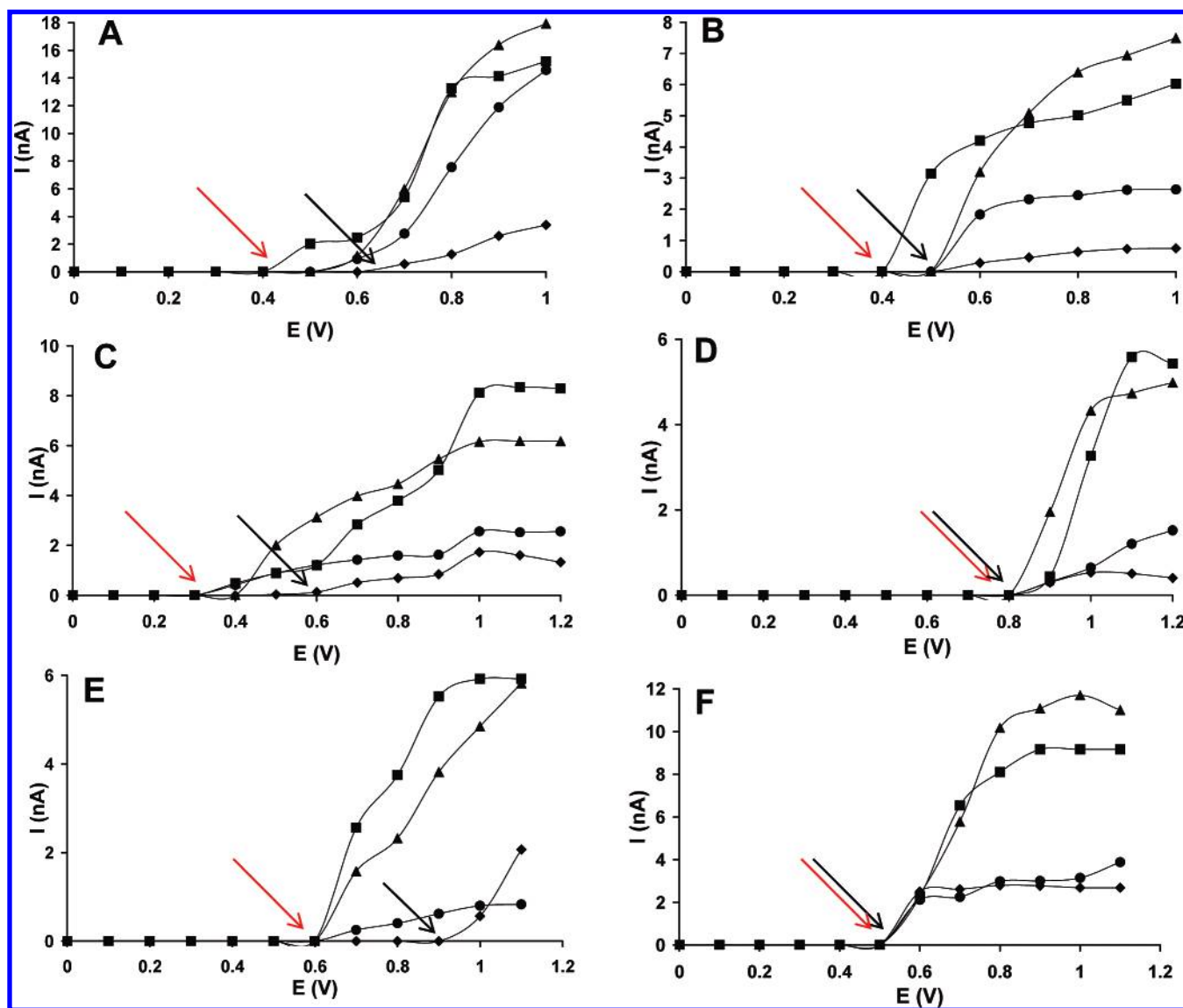


Figure 1. Hydrodynamic voltammograms of different compounds obtained from each kind of electrode studied. ■, SPE-MWCNT-A; ▲, SPE-MWCNT-B; ●, SPE-SWCNT; ◆, SPE bare. (A) Arbutin, (B) rutin, (C) ascorbic acid, (D) folic acid, (E) maltol, and (F) vanillin.

Edmond, Canada) and consisted of a glass plate (88 mm × 16 mm) with a four-way injection simple cross, a 74-mm-long separation channel, and side arms measuring 5 mm long. The dimensions of the channels were 20 μm in depth and 50 μm wide. The amperometric detector (end-channel detection) consisted of an Ag/AgCl wire as a reference electrode, a platinum wire as a counter electrode, and a carbon thick-film screen-printed electrode (0.3 × 2.5 mm) as a working electrode. These electrodes were provided by Dr. Wang's laboratory (Arizona State University³⁰). We introduced a spacer (easily removable adhesive tape; 60 μm) between the electrode surface and the channel outlet to control the distance between the electrode and the channel separation with reproducibility.

The working electrode was modified by casting 2 μL of DMF containing 1 mg/mL carbon material and allowing it to dry at room temperature for 12 h.³¹

Amperometric detection was performed using a Potentiostat Autolab PGSTAT12 from Eco Chemie. The high-voltage power

supply (from Glassman High Voltage, Inc.) had an adjustable voltage range between 0 and +5000 V.

Procedures. Standards. All stock solutions of standards were dissolved in MeOH and then stored at -20°C except folic acid, which was dissolved in a solution of 1 M NaOH. Working solutions for the studies were appropriately diluted in the electrophoresis buffer. All working solutions were well protected from light and used within 24 h after preparation.

Food Sample Preparation. Formulation Samples. Ten tablets of Becozyme (containing 10 mg of pyridoxine and 200 mg of ascorbic acid per tablet and other vitamins) were pulverized and added into a recipient. About 50 mg of this powder was introduced into a glass flask and 25 mL of phosphate buffer (10 mM pH 6) was added, previously bubbled with N_2 for 30 min. The sample was totally dissolved. A suitable aliquot was taken for analyses.

Vanilla Pod Samples. A 0.2 g sample of vanilla bean was finely chopped and macerated for 12 h at 40°C with 2 mL of water in a closed vessel. Ethanol (2 mL) was added, and the mixture was mixed thoroughly and macerated again for 3 days. The mixture

(31) Pumera, M.; Merkoci, A.; Alegret, S. *Electrophoresis* **2006**, *27*, 5068–5072.

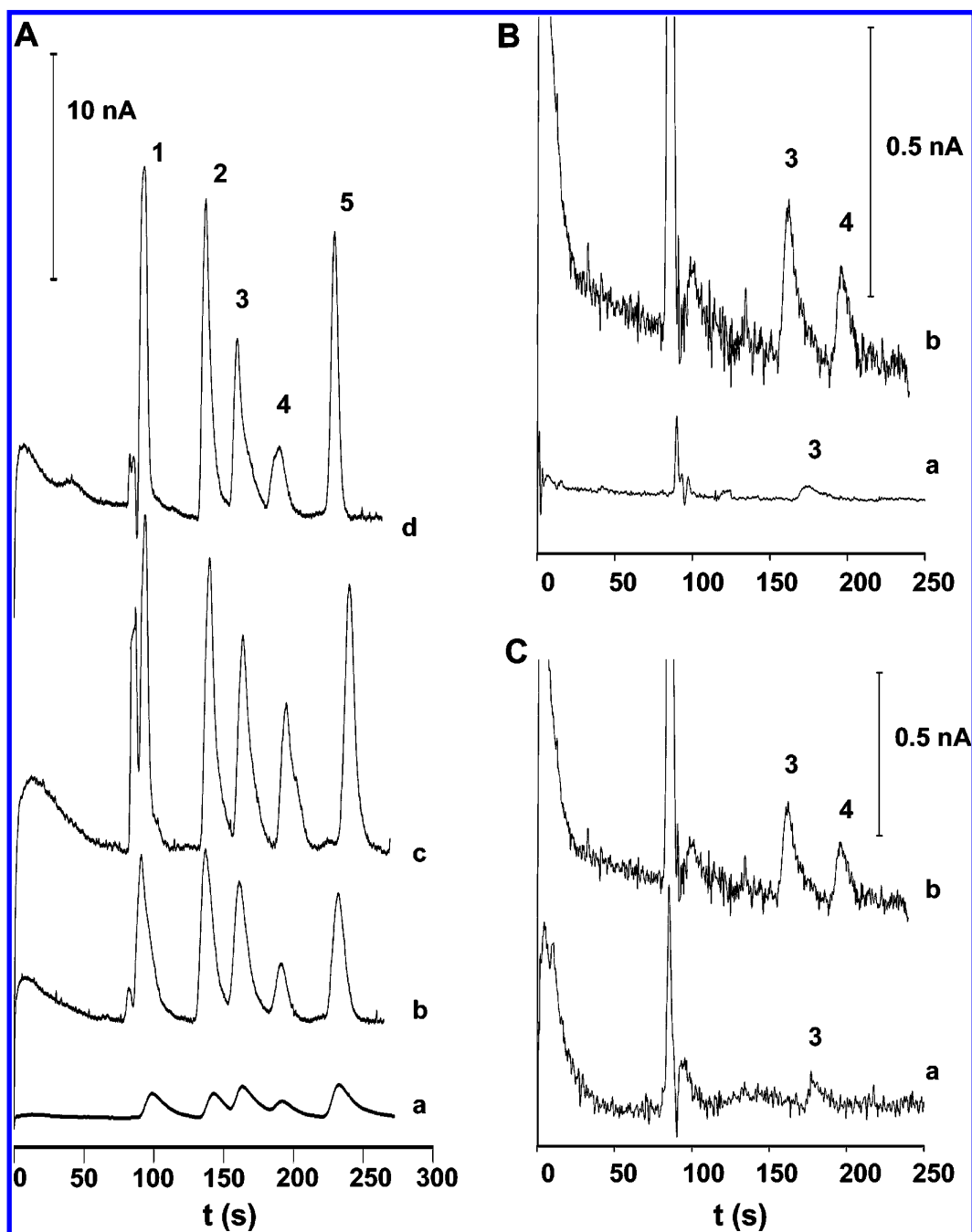


Figure 2. (A) Microchip electropherograms corresponding to a mixture of antioxidant standards with electrode materials studied: (a) SPE bare, (b) SPE-SWCNT, (c) SPE-MWCNT-B, and (d) SPE-MWCNT-A. (B) Electropherograms corresponding to extract from apple peels: (a) SPE bare, (b) SPE-MWCNT-A. (C) Analysis of apple extract with SPE-MWCNT-A: (a) pulp, (b) peel. Peaks: (1) arbutin, (2) phloridzin, (3) catechin, (4) rutin, and (5) ascorbic acid. Conditions: borate buffer 50 mM pH 9, separation voltage 2 kV, injection voltage 2 kV for 5 s, detection voltage 1 V, with 1 M HNO₃ in the detection reservoir.

was poured through a sintered filter funnel and the filtrate collected (adapted from ref 32).

Apple Peel and Pulp Samples. Apple was peeled to separate pulp and peel. Five grams of each was extracted, separately, with 10 mL of methanol for 1 h and 30 min. Samples were centrifuged at 13706g for 10 min (adapted from ref 33).

A secondary standard referenced in the Drug Master File (SW/1211/03) was used as reference material (property of Exxentia);

a certain amount was weighed to contain ~2 mM total isoflavones. A 20-mL volume of ethanol was added to the sample, and the resultant mixture was sonicated for 30 min. Samples were centrifuged for 10 min at 2000g in order to eliminate insoluble matter.

All samples were protected from the light and used within 24 h after preparation.

Electrophoretic Procedure. The channels of the glass microchip were treated before use and between groups of runs by rinsing them with 0.1 M NaOH and deionized water for 15

(32) Herrmann, A.; Stöckli, M. *J. Chromatogr., A* **1982**, *246*, 313–316.

(33) Escarpa, A.; González, M. C. *J. Chromatogr., A* **1998**, *823*, 331–337.

min each. This procedure was carefully monitored to obtain reproducible results. The optimum electrophoresis buffer consisted of 10 mM phosphate buffer (pH 7) for vitamins, 50 mM borate buffer (pH 9) for polyphenols, 20 mM borate buffer (pH 9.5) for flavors, and 10 mM borate buffer (pH 8) for isoflavones. The running buffer and sample reservoirs were filled with the respective solution. The detection reservoir was filled with a 1 M HNO₃ solution. A voltage of 2000 V was applied for 5 min to the buffer reservoir to fill the separation channel while the detection reservoir was grounded and the others were floating. This process was performed on each sample reservoir for 20 s to facilitate filling the injection channel (between the separation channel and the sample reservoir), and then the voltage was applied for 5 min to the running buffer reservoir to eliminate the remains of the previously introduced samples from the separation channel.

Amperometric Detection. A detection voltage of +1.2 V was applied to the working electrode for vitamin and isoflavone analysis, +0.9 V for flavors, and +1.0 V for polyphenols. All experiments were performed at room temperature.

Safety considerations. *The high-voltage supply should be handled with extreme care in order to avoid electrical shock.*

RESULTS AND DISCUSSION

In order to study the effect of the CNTs on the voltammetric response and to find the suitable detection potential, hydrodynamic voltammograms (HDVs) using both unmodified (SPE) and CNT-modified electrodes (SWCNT, MWCNT-A, MWCNT-B) were constructed. Figure 1 shows selected HDV profiles for each analyte group (polyphenols, vitamins, flavors, isoflavones) where two surface effects were observed: the lowering of oxidation potential and the enhancement of voltammetric current.

For dietary antioxidants (polyphenols), their oxidation in the presence of CNTs always started at substantially lower potential (about +0.4 or +0.5 V), decreasing the oxidation potential by ~0.3 V when CNT-modified electrodes with respect to use of bare SPE. Figure 1A,B shows the HDVs corresponding to two very different phenolic structures (arbutin and rutin) where the CNT surface effect is easily observed. On the contrary, while a profound lowering of potential effect was noticed for vitamin C (Figure 1C), the oxidation potential obtained for B-group vitamins (B₆ and folic acid) was not lower than +0.8 V with independence of the CNT material explored (Figure 1D). With respect to vanilla flavors and flavor enhancers, malthol (Figure 1E) and ethyl maltol (HDV not shown) showed notably a lower potential with oxidation starting at +0.6 V instead of +0.9 V when CNTs were used while neither vanillin (HDV not shown) nor ethyl vanillin (Figure 1F) showed this effect. The lowering of the potential of genistein from +0.9 to +0.7 V (HDV profiles not shown) was also observed.

The CNTs have a much larger specific surface area, so that current density is low and overpotentials are also lowered. That real effect was exhibited mainly when MWCNT materials were used, with independence of the analyte under study. The whole of the results revealed the complexity of the target analyte mixtures since not all compounds involved in each group exhibited the same electrochemical behavior. On the basis of all HDVs obtained, a relationship between this surface area effect and structures of the analytes was observed. Decreasing of the oxidation potential happened only on the analytes with enol and enediol as electroactive groups (polyphenols, ascorbic acid,

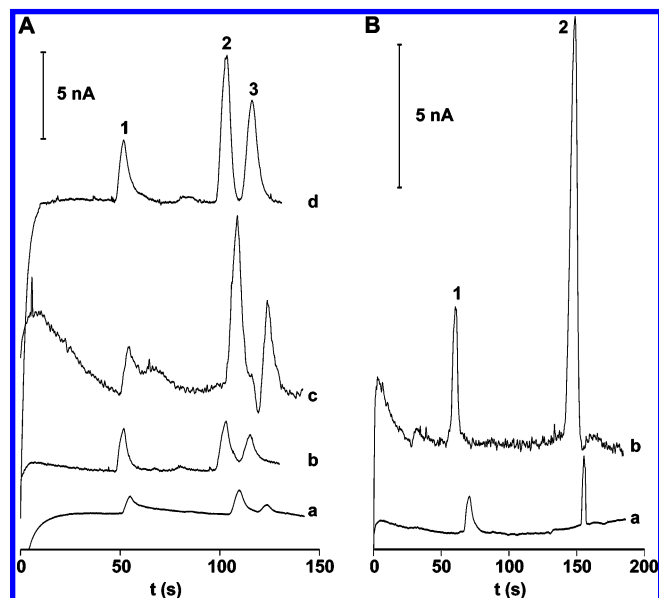


Figure 3. (A) Microchip electropherograms corresponding to a mixture of water-soluble vitamin standards with each kind of electrode studied: (a) SPE bare, (b) SPE-SWCNT, (c) SPE-MWCNT-B, and (d) SPE-MWCNT-A. (B) Determination of vitamins in a pharmaceutical supplement (Becozyme): (a) SPE bare and (b) SPE-MWCNT-A. Peaks: (1) pyridoxine, (2) vitamin C, and (3) folic acid. Conditions: phosphate buffer (10 mM pH 7), separation voltage 2 kV, injection voltage 2 kV for 3 s, detection voltage 1.2 V, with 1 M HNO₃ in the detection reservoir.

isoflavones, maltol). On the contrary, this effect was not observed for vanillin and pyridoxine containing carbonyl and hydroxymethyl as electroactive groups, respectively.

Analytical Merits of Carbon Nanotubes in Qualitative Analysis Domain. As we have previously mentioned, voltammetric profiles indicated a second important surface effect: CNT materials involved (SWCNT, and mainly MWCNT-A and MWCNT-B) offered a greatly improved sensitivity. Figure 2A shows the electropherograms obtained for dietary antioxidant standards at all CNT materials used where an enhancement of sensitivity with independence of CNT material used was clearly observed. Sharp and well-resolved peaks of four polyphenols (arbutin, phlorizin, (+)-catechin, rutin) and ascorbic acid were obtained in contrast with tailing unresolved peaks of these analytes using unmodified SPE. Since MWCNT-A gave the best sensitivity and the lowest noise, it was chosen as a material optimum for the analysis of antioxidants in food samples. Figure 2B,C shows selected examples of apple samples (peel and pulp). Figure 2B shows the surface area effect observed during the detection of (+)-catechin and rutin from apple peels when MWCNT-A was used. When bare SPE was used only a smoothly tailing peak for (+)-catechin was observed (electropherogram a). These results revealed the suitability of the modified electrodes for detection of both antioxidants. In addition, Figure 2C shows the electropherograms obtained for peel and pulps when MWCNTs-A was used. As expected, rutin was only detected in peels (this analyte is a fingerprint of these matrixes (Figure 2C, b) indicating again the suitability of the CNTs as novel materials in antioxidant detection. This fact could be very important in controlling adulteration of apple juices using peels in their elaboration.

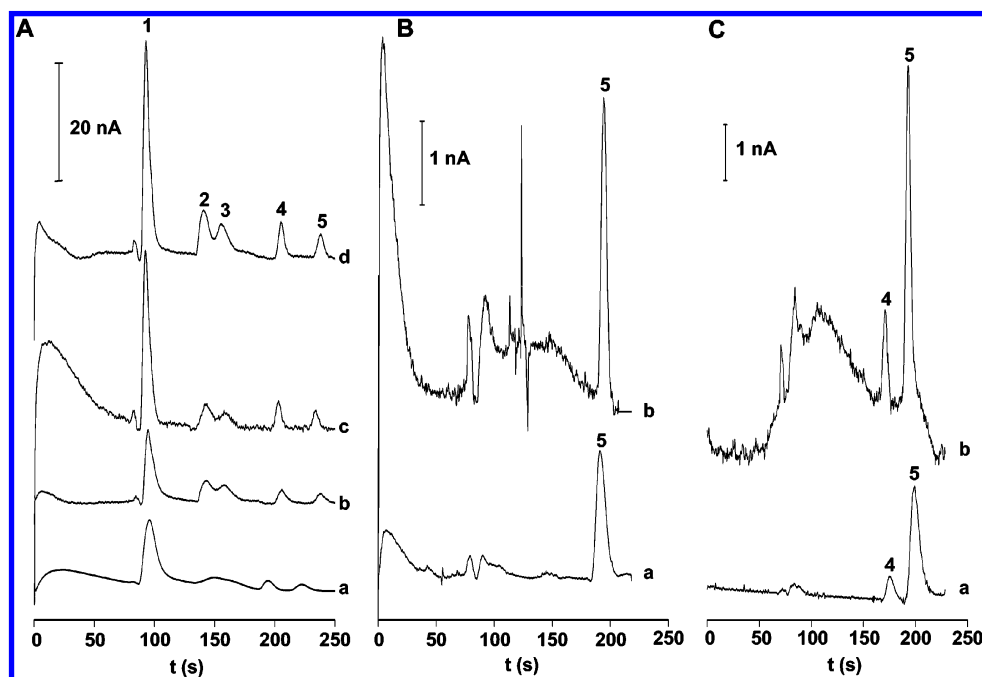


Figure 4. (A) Microchip electropherograms corresponding to a mixture of flavor standards with each kind of electrode studied: (a) SPE bare, (b) SPE-SWCNT, (c) SPE-MWCNT-B, and (d) SPE-MWCNT-A. (B, C) Analysis of vanilla beans. Electropherograms: (B) natural vanilla extract, (C) spiked with ethyl vanillin. (a) SPE bare and (b) SPE-MWCNT-A. Peaks: (1) vanillic alcohol, (2) ethyl maltol, (3) maltol, (4) ethyl vanillin, and (5) vanillin. Conditions: borate buffer (pH 9.5, 20 mM), separation voltage 2 kV, injection voltage 2 kV for 3 s, detection potential +0.9 V, with 1 M HNO₃ in the detection reservoir.

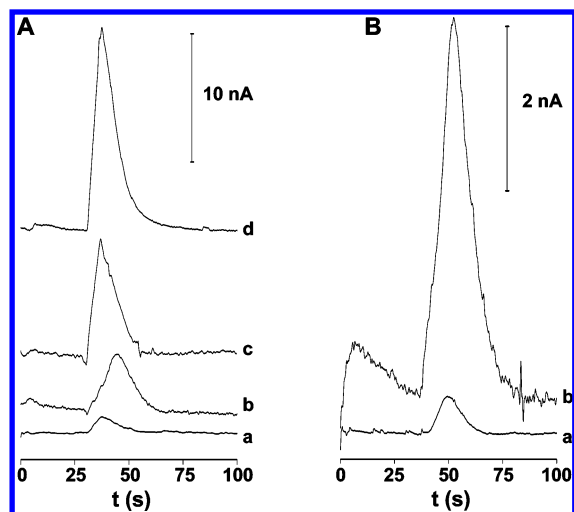


Figure 5. (A) Microchip electropherograms corresponding to genistein standard with each kind of electrode studied: (a) SPE bare, (b) SPE-SWCNT, (c) SPE-MWCNT-B, and (d) SPE-MWCNT-A. (B) Analysis of a total isoflavones in a reference sample (a) SPE bare and (b) SPE-MWCNT-A. Conditions: borate buffer 10 mM pH 8, separation voltage 2 kV, injection voltage 2 kV for 6 s, detection voltage +1.2 V, with 1 M HNO₃ in the detection reservoir.

Figure 3 shows the separation of prominent B group and C vitamin standards (A) in connection with their analysis in a selected formulation (B) using bare SPE and a CNT-modified electrode. In this case, a profound enhancement of the sensitivity was noticed when MWCNTs were used in the detection of standards. MWCNT-A was chosen as optimum material for vitamin detection in formulations showing an enhancement of the sensitivity in the analysis of vitamins B₆ and C in the formulations examined (see Figure 3B).

Table 1. Resolution Obtained for Analytes Couples of Food Significance

food group	analyte couple	resolution		
		SPE bare	SPE-MWCNT-A	ratio ^a
polyphenols	phloridzin-(+)-catechin	0.8	1.9	2.3
	(+)-catechin-rutin	1.0	2.1	2.1
vitamins	vitamin C-folic acid	1.8	1.4	const ^b
vanilla flavors	maltol-ethyl maltol	0.6	1.0	1.7
	vanillin-ethyl vanillin	1.7	2.9	1.7
	<i>vanilla sample^c</i>	1.1	2.4	2.2

^a Ratio was calculated as resolution obtained using SPE-MWCNT-modified electrode/resolution obtained using SPE-bare electrode. ^b Resolution ratio obtained was 0.8 and understood as constant. ^c Italic type indicates that this resolution was obtained during analysis in vanilla samples.

Figure 4A shows the enhancement of sensitivity observed when CNT materials (b–d) were used as modifiers of SPE bare (a) electrodes in the detection of vanilla flavors and related compounds. To illustrate the power of CNT in the detection of frauds, natural vanilla samples were also analyzed (Figure 4B,C). The enhancement of sensitivity was also clearly noticed during the analysis of natural vanilla samples (Figure 4B) when MWCNT-A was used as optimum material (electropherogram 5B, b). Under these high sensitivity conditions, ethyl vanillin was not detected while vanillin was really detected. This result confirmed the natural origin of the vanilla pod examined and indicated the suitability of these detectors for controlling their authenticity. Also, vanilla extract was fortified in ethyl vanillin in order to evaluate the capability of the method to detect a possible adulteration in vanilla samples using again both MWCNT-A modified and bare electrode (Figure 4C). The enhancement of sensitivity was also

Table 2. Selected Analytical Information Obtained from Sample Analysis

food group	analytes	SPE bare			SPE-MWCNT-A			slope ratio ^b
		$w_{1/2}$	S/N	LOD ^a (μ M)	$w_{1/2}$	S/N	LOD ^a (μ M)	
polyphenols	(+)-catechin	11.8	4	4	8.2	6	2	9
	rutin	nd ^c	nd	23	8.6	4	12	11
vitamins	pyridoxine	4.8	83	29	4.0	15	8	16
	vitamin C	2.5	159	11	5.6	50	11	4
vanilla flavors	ethyl vanillin	7.9	25	15	5.3	8	7	9
	vanillin	9.4	180	12	5.4	58	7	7
total isoflavones	genistein	11.3	63	1	14.3	76	0.8	8

^a LODs were calculated as $S/N = 3$ criteria. ^b Determined as the ratio between the calibration slope obtained using SPE-MWCNT-A/calibration slope obtained using SPE bare ($r > 0.990$ for all calibration curves studied). ^cnd, not detected

clearly observed during the detection of the ethyl vanillin–vanillin target couple.

“Total isoflavones”⁹ (genistein and daidzein and their related glycosides genistin and daidzin) form a very important group of compounds, which possess various health-promoting functions and form a group that occurs naturally in soybeans. In the analysis of isoflavones, the differentiation of their possible forms seems to be sometimes irrelevant because only aglycons are absorbed in the human gastrointestinal tract, the most important information being the “total isoflavones” content. Figure 5 shows the enhancement of sensitivity observed when CNT materials were used as modifiers of SPE electrodes in the detection of both genistein (A) (as representative prominent isoflavone standard) and nonseparated total isoflavones in the reference material (B).

In all food analytes of significance examined before, both the sharp and well-resolved peaks using MWCNT-A-modified electrodes indicated the faster electron transfer that resulted in smaller values of the half-peak widths and had as a clear consequence the improvement of resolution during the analysis of the solid samples. Table 1 lists the resolution obtained for each target analyte couple when unmodified (SPE) and modified (MWCNT-A) samples were used, as well as the resolution ratio obtained between them. While no significant improvement of resolution peak to peak (i.e., vitamin C to folic acid) was obtained (from 1.8 to 1.4), the resolution of all analyte couples involved was improved (for example, malthol–ethyl malthol resolution was improved from the unacceptable value of 0.6 to the acceptable one of 1.0). Interestingly, ethyl vanillin–vanillin resolution was also improved during the analysis of natural vanilla from 1.1 to 2.4, revealing the analytical power of these materials in food sample analysis. In order to interpret these data suitably, the resolution ratio was also calculated (between resolutions obtained with modified and unmodified detectors), finding that the improvement factor of resolution was constant and around 2 with independence of the analytes studied. It opens new directions in the area of complex separations where many analytes could constitute the final composition.

Table 2 summarizes the relevant information obtained from food sample analysis when MWCNT-A was used as optimum material. As has been reported in other standards of clinical and environmental significance, half-peak widths were also dramatically decreasing in food analyte standards (i.e., half-peak widths for arbutin, phloridzin, (+)-catechin, rutin, and ascorbic acid standards: 5.6 vs 15.2; 6.1 vs 14.3; 6.7 vs 16.7; 10.7 vs 17.6; 5.7 vs

15.5 s), and, interestingly, it was also noticed in the food sample analysis (see Table 2). Indeed, during the detection of samples of complex flavonoids as well as during the detection of the ethyl vanillin–vanillin couple, half-peak widths were also decreased while no significant decrease of half-peak widths was obtained when water-soluble vitamins and total isoflavones were really detected in the samples. Signal-to-noise ratio and estimated LODs were also good during analysis, allowing the detection of analytes. In general, LODs were of micromolar range and were also improved when modified detectors were used for antioxidants (2 vs 4 μ M 12 vs 23 μ M (+)-catechin and rutin, respectively; 8 vs 30 μ M for pyridoxine) as well as vanilla flavors (12 vs 7 μ M).

The enhancement of sensitivity was also coupled to the resistance of the surface fouling and hence to good stability. While signal at bare SPE was decreased to 20% for some polyphenols, 80% of the total signal was kept for selected analytes after repetitive injections ($n = 10$) when optimum material was used (MWCNT-A).

Analytical Merits of MWCNT in Quantitative Analysis Domain. Quantitative analysis is very important in food analysis. Several quality and security controls are based on accurate measurements of concentration of target analyte. In this way, it is very important to explore the behavior of CNTs in this domain of the analysis. Analytical performance of the MWCNT-A detector as optimum material in terms of precision was studied, and calibration plots were obtained for all analyte groups. Precision was evaluated in the analytical signals obtained from selected analytes with RSDs values of less than 10% for polyphenols, 11% for vitamins, 9% for flavors, and 3% for isoflavones. In addition, calibration plots were performed using both unmodified (SPE) and modified detectors (MWCNT-A), and good linear correlations were obtained in all cases examined ($r > 0.990$). The enhancement of sensitivity was rigorously evaluated from the calibration slope ratio between MWCNT-A and bare SPE (see also Table 2). An impressive slope ratio ranging from 4- to 16-fold was observed for the selected analytes, quantitatively indicating the improvement of analytical sensitivity of CNT materials.

As we have previously mentioned when describing the problematic analysis of isoflavones, a total amount of isoflavone aglycons (total isoflavones) could provide sufficient information when different soy products are considered for consumption. In addition, analysis of total isoflavones assures the quality of the products, such as pharmaceuticals or food. Consequently, the fast and reliable quantitation of total isoflavones should become

Table 3. Reliability of the Methods and Quantitative Data Using MWCNT-A as New Detectors in Food Analysis

sample	analytes	level	precision (RSD (%))	reference value	accuracy (%)	remarks
secondary std	total isoflavones	43.6 ± 1.4 (%)	3	45.8 (%)	5	accurate and traceable determination
apple	(+)-catechin	0.25 mg/100 g	nd	na ^a	nd	quantitative data found in agreement with relevant literature
formulation	rutin pyridoxine	1.9 mg/100 g 9.6 ± 0.6 mg/tablet	6	10 mg/tablet	4	comparison of quantitative data obtained with those given by manufacturer. accurate determination
vanilla pod	vitamin C	182 ± 8 mg/tablet	4	200 mg/tablet	9	excellent recovery (close to 100%) quantitative found data in agreement with relevant literature
	ethyl vanillin	recovery 101 ± 3(%)	3	100%	1	
	vanillin	60 ± 3 mg/g	5	na	nd	

^a na, not available.

essential in food–drug quality control of market production. In this way, the reliability (precision and accuracy) of the proposed new MWCNT detectors in the quantitative domain was rigorously evaluated using a reference material (a secondary standard from Drug Master File SW/1211/03; containing 45.8% (w/w) (indicated for the secondary standard). As can be observed, the precision (RSD less than 3%) and accuracy obtained (systematic error of 5%) were excellent, indicating a very good reliability of these materials coupled to microfluidic platforms in quantitative domains. Because of this performance, MWCNT-A was extended to the other samples with quantitative purposes. Table 3 also lists the quantitative analyte levels as well as the evaluation of the precision and accuracy (where appropriate) obtained in the analysis from the food samples examined. The results obtained in all cases studied confirmed the suitability of nanotube detectors in the quantitative domain.

CONCLUSION

Carbon nanotube-modified SPE detectors coupled in microfluidic chips are a new and promising material for electrochemical sensing of food analytes of significance in both qualitative and quantitative domains. These unique materials allowed the decrease

of the overpotential, enhancing sensitivity, and improving the resolution in the detection of dietary antioxidants, water-soluble vitamins, vanilla flavors, and total isoflavones during their analysis in food samples. In addition, very good accuracy was also obtained when these materials were used in the analysis of referenced samples with high traceability. These materials coupled to the well-established advantages of the first-generation CE microchips are going to break some frontiers in the near future advancing clearly toward the “food lab-on-a-chip” concept.

ACKNOWLEDGMENT

Financial support from the Ministry of Science and Technology (Spain) CTQ2005-09079-C03-03 is gratefully acknowledged. A.G.C. acknowledges the fellowship received from the Spanish Ministry of Education and Science. M.P. is grateful to the Japanese Ministry for Education, Culture, Sports, Science and Technology (MEXT) for funding through the ICYS program.

Received for review June 13, 2007. Accepted July 24, 2007.

AC071247I