Research Article

Evaluation of carbon nanostructures as chiral selectors for direct enantiomeric separation of ephedrines by EKC

Single-walled nanotubes and multi-walled nanotubes (MWNTs) have been evaluated as chiral selectors for the enantiomeric separation of ephedrines by using EKC with surfactant-coated carbon nanotubes. The analysed compounds were (+)-ephrine, (+)-norephedrine and (+)-N-methylephedrine. The potential of those carbon nanostructures as chiral selectors has been evaluated by changing different experimental variables such as pH, addition of organic modifiers, potential and injection time. The capability of MWNTs to resolve enantiomeric mixtures was demonstrated by using partial filling of the capillary with concentrated surfactant-coated MWNTs. Differences in the enantioselectivity were discussed.

Keywords: Carbon nanostructures / Chiral separation / Ephedrines

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1 Introduction

Ephedrines are potential stimulant drugs of the central nervous system that are widely used in many pharmaceutical applications. Concretely, ephedrine, norephedrine and N-methylephedrine exist as their corresponding (+)-enantiomers, which differ in their pharmacological and toxicological effects. Therefore, enantiomeric analysis is a topic of interest in pharmaceutical, toxicological and clinical analysis.

Direct enantiomeric analysis by EKC has been widely studied in the last years [1–4], taking into account the advantages of this technique, namely expeditiousness, high resolution as well as efficient separation and low consumption of sample and reagent volumes. In EKC, the enantiomeric separation is achieved by adding a chiral selector to the pseudostationary phase. Newly available chiral selectors have been deeply reviewed by Eeckhaut and Michotte [5]. CDs are commonly added as chiral selectors to pseudostationary phases [6, 7]. Chiral separations by microemulsion EKC (MEEKC) using chiral pseudostationary phases (water-immiscible compound, chiral surfactant) have also been proposed [8, 9]. An excellent review on the topic has been recently published [10].

The use of nanoparticles as pseudostationary phase in EKC was first proposed in 1989 by Wallingford and Ewing [11]. Recently, Wang et al. [12] have used carboxylated single-walled nanotubes (SWNTs) as carriers for CE. Carboxylated multi-walled nanotubes (MWNTs) have also been described to enhance the separation of purine and pyrimidine bases [13]. The use of silica, polymers, molecularly imprinted polymers, gold nanoparticles as well as dendrimers and polymeric surfactants as pseudostationary phases have been recently reviewed [14]. The applicability of carbon nanostructures (fullerenes and carbon nanotubes) has not been considered in this article, however. Enantiomeric separations using nanoparticles based pseudostationary phases have also been described in the last years. With this aim, molecularly imprinted polymers and molecular micelles have been used [14].

Our research group has recently developed and proposed a new EKC modality, using surfactant-coated carbon nanotubes (SC-CNTs) [15] in which SWNTs are dispersed into a micellar media to obtain the SC-CNTs. Dispersion is carried out by sonication to obtain a homogeneous phase, stable under high electric field and compatible with the detector. After the dispersion procedure, SC-CNTs are added to the BGE. EKC with SC-CNTs can be also combined with partial filling. Partial filling was first introduced by Valtcheva et al. [16] and subsequently modified by Tanaka and Terabe [17]. In this technique, the capillary is partially filled with a separation solution generating a separation zone, with a length of ca. 2/3 the separating zone is two thirty parts of the total
length of the capillary. Particularly, partial filling with SC-CNTs is based on the introduction of a small volume (similar to that of the sample plug) of concentrated SC-CNTs in order to increase the electrochromatographic resolution due to π–π interaction between the CNT’s side wall and the analyte without disturbing the baseline. The principal difference between partial filling with SC-CNTs and conventional partial filling [16–18] or the recently published full filling approach [19] is the lower length of the inserted plug.

Since the discovery of carbon nanotubes, [20] their chirality (chiral angle between hexagons and the tube axis, together with the diameter) has been investigated because of its great potential for isomer separation. From the symmetry point of view, SWNTs can be either achiral or chiral [21, 22]. MWNTs also show chirality although in this case, its definition is more complicated as it will be later commented on [23, 24]. Although, in a theoretical study, it has been reported that the chiral nanotubes are not able to separate enantiomers [25] on the basis of an internal molecule–nanotube interaction, Heller et al. [26] demonstrated that the sonication of dispersions of nanotubes allowed modifications in the chiral properties of these nanostructures, inducing the generation of some chirality in achiral nanotubes. From the above, the possibility of using carbon nanostructures as chiral selectors for enantiomeric separations seems obvious.

The aim of the present paper was to evaluate the potential of carbon nanotubes as chiral selectors in CE as a first approach for the direct resolution of enantiomeric mixtures of (±)-ephedrine, (±)-norephedrine and (±)-N-methylephedrine. For this purpose SC-CNTs were used as the pseudostationary phase and partial filling was selected as a key point for method development. In addition, ultrasones have been identified as a critical way to introduce chirality in CNTs. The variation in the enantioselectivity was studied as a function of the experimental conditions.

2 Materials and methods

2.1 Apparatus

A Beckman Coulter (Palo Alto, CA, USA) P/ACE 5500 CE system equipped with a DAD was used to separate and quantify the analytes. CE separations were accomplished by using a fused-silica capillary (75 μm id) with an effective length between inlet and detector of 50 cm (total length of 57 cm).

2.2 Chemical

Single-walled carbon nanotubes with diameters between 0.7 and 1.2 nm and lengths 2–20 μm, and multi-walled carbon nanotubes with diameters between 6 and 20 nm and 1–5 μm length were obtained from Mer Corporation (Tucson, AZ, USA). All the carbon nanostructures were achiral, according to the specification of the distributor. (±)-Ephedrine, (±)-ephedrine, (±)-norephedrine, (±)-norephedrine, (±)-N-methylamphetamine, (±)-N-methylamphetamine and SDS were purchased from Sigma Aldrich (Madrid, Spain). Ethanol, 2-butanol, ACN, hexane and boric acid were supplied by Panreac (Barcelona, Spain). The pH of the electrophoretic buffer was adjusted with 1 M NaOH and 1 M HCl aqueous solutions. Stock standard solutions of the individual drugs were prepared at a concentration of 100 mg/L by dissolving the accurately weighed amount in Milli-Q water. Working standard solutions were prepared on a daily basis by rigorous dilution of stocks in Milli-Q water. Migration times of individual enantiomers were identified by spiking mixtures of all the compounds with the corresponding enantiomer.

2.3 Electrophoretic conditions

The first step was the dispersion of carbon nanotubes following a previously optimized procedure [27]: 1 mg of SWNTs or MWNTs was mixed with 25 mL of 17.3 mM SDS and sonicated (50 W, 60 Hz) for 20 min to obtain a final carbon nanotube concentration of 40 mg/L. The background electrophoretic buffer used to achieve the chiral separation of (±)-ephedrine, (±)-norephedrine and (±)-N-methylamphetamine was 20 mM boric acid, 20 mM SDS and 15% v/v ACN adjusted to pH 9 with 1 M NaOH. Once prepared, a volume of 1.2 mL of dispersed carbon nanotube solution was added to 15 mL of buffer to obtain a final concentration of 3.2 mg/L as limited for baseline stability [15]. Samples were injected hydrodynamically at 0.5 psi for 10 s. The study of partial filling was performed by injecting (10 s at 0.5 psi) surfactant-coated MWNTs (SC-MWNTs) (6.4 mg/L) before the sample injection.

In all the cases, the temperature of the capillary was set at 20°C and the applied voltage for the electrophoretic separation was 15 kV. The wavelength was fixed at 200 nm.

Initially, the capillary was sequentially conditioned by flushing 1 M HCl, Milli-Q water, 0.1 M NaOH and Milli-Q water for 5 min, and finally with the running buffer for 15 min. Between runs, the capillary was flushed sequentially at 20 psi with 0.1 M NaOH (2 min), Milli-Q water (2 min) and the electrophoretic buffer (2 min).

3 Results and discussion

The potential of single-walled and multi-walled carbon nanotubes as chiral selectors was evaluated in terms of resolution R, and enantioselectivity (αenant) [1].

Ephedrine and norephedrine are compounds that exist as pair of enantiomers, and in the absence of chiral selectors in the electrophoretic buffer no separation between enantiomers exists. By way of example, Fig. 1A shows the electropherogram obtained for a standard solution of (+)- and (−)-ephedrine, and (+)- and (−)-norephedrine (10 mg/L each).
Figure 1. (A) Electropherogram obtained for a standard solution of enantiomers (10 mg/L each) without SC-CNTs. (B) Electropherogram obtained for a standard solution of the enantiomers (5 mg/L each) with SC-SWNTs as chiral selector. 1, (-)-norephedrine; 2, (+)-norephedrine; 3, (-)-ephedrine; and 4, (+)-ephedrine. Experimental conditions: 20 mM boric acid, 20 mM SDS and 15% v/v ACN adjusted at pH 9, voltage 15 kV and injection time 10 s.

The electropherogram was obtained under the same instrumental conditions as described in Section 2.3, but without using SC-CNTs.

3.1 Evaluation of SC-SWNTs as chiral selectors

In this paper, the optimized procedure for CNTs dispersion proposed by our research group was applied using an SWNT concentration of 3.2 mg/L in the electrophoretic buffer. Figure 1B shows the electropherogram obtained for a mixture of four analytes (experimental conditions indicated in the figure caption). As can be seen, the enantiomers of ephedrine showed some tendency to be separated ($R_s = 0.97$ and $\alpha_{enant} = 1$) whereas norephedrine enantiomers overlapped. If we consider that some interaction between the analytes and the nanotube’s surface exists, the incipient separation of ephedrine enantiomers was probably due to chiral interactions between the analytes and the chirality induced on the nanotube’s surface after sonication. In the case of norephedrine, these interactions were not strong enough to separate both enantiomers.

The chemical variables potentially influencing the electrophoretic separation were evaluated for enantiomers resolution. Taking into account the effect of ultrasounds on nanotubes’ chirality, the sonication time was studied over the interval 20–60 min. As longer times did not improve chiral separation, 20 min was selected as the optimum time.

The variations of pH (from pH 7.5 to 9.8), applied voltage (10, 15 and 20 kV), addition of organic modifiers (ethanol and 2-butanol) and the reduction of injection time from 10 to 5 s did not improve the chiral separation either for ephedrine or for norephedrine enantiomers. Therefore, in order to increase the interaction between ephedrines and carbon nanotubes, maintaining the baseline stability, partial filling of the capillary with a plug of concentrated SC-SWNTs (6.4–10 mg/L) was studied [15]. Under these conditions, chiral interaction should be favored. However, in this particular case, the electropherograms obtained showed an increase in the background noise with negligible influence on peak resolution. Therefore, the SWNTs employed in this work are not useful as chiral selector for enantiomeric separation of the ephedrines selected.

3.2 Evaluation of SC-MWNTs as chiral selectors

Multiwalled carbon nanotubes can consist of concentric cylinders of SWNTs (nested tubes) or a single sheet of graphene rolled around itself (scroll structure). The chirality of the MWNTs strongly depends on the arrangement of the walls (nested tubes or scrolls), which can be related to the growing mechanism followed. Generally speaking, it can be stated that the scroll structures present a uniform chirality while for nested tubes, a random distribution of chirality of each concentric layer can generate MWNTs with different chirality [24, 28]. Although no information about inducing chirality by sonication in MWNTs has been reported, we studied the effect of ultrasounds for introducing chirality on MWNTs following the procedure described in Section 2.3 and using a working solution of 3.2 mg/L of MWNTs in the electrophoretic buffer. The increase in the migration times suggests that there was an interaction between the analyte and the pseudostationary phase (see Table 1), although no enantiomeric separation was observed. Therefore, the experimental conditions were modified in order to achieve...
Table 1. Separation data obtained for MWNTs as chiral selectors

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a) Mixture of (+)-norephedrine.
b) Migration time for (–)-norephedrine.
c) Migration time for (+)-norephedrine.

the maximum separation. As it was the case of SWNTs, variations in pH (from 7.5 to 9.8), applied voltage (10, 15 and 20 kV), injection time (5 and 10 s), or an increase in the sonication time did not improve those results. Therefore, partial filling was included in the procedure by using 6.4 mg/L SC-MWNTs solution, sonicated for 20 min. The injection time was fixed at 10 s as it has negligible effect on separation or resolution and the voltage was set at 15 kV. For simplicity, norephedrine enantiomers (prepared at individual concentration of 5 mg/L) were selected as test analytes to optimize the variables affecting the electrophoretic separation. The first variable evaluated was the pH of the BGE (20 mM boric acid, 20 mM SDS, 15% ACN and 3.2 mg/L MWNTs) between 8.5–9.8. The marked increase in the migration time for norephedrine demonstrated a strong interaction between the analyte and the SC-MWNTs. In spite of this interaction, the values of the resolution factor suggested that there was no enantiomeric resolution at pH 8 and 9.8 while a low chiral interaction has observed at pH 8.5 (see Table 1). However, the values of $R_s$ at pH 9.0 demonstrated that the enantiomeric resolution of norephedrine isomers can be achieved. Concerning enantioselectivity, similar values were obtained in all cases and thus pH 9 was selected to study the effect of the presence of organic modifiers in the BGE in the enantiomeric separation.

The presence of an organic modifier in micellar EKC modifies the behavior of the analytes within the electrophoretic capillary, affecting the resolution positively through the enhancement of the solubility capacity and increasing the migration time, which can positively affect the analytes resolution. Indeed, short chain alcohols can act as cosurfactants making the microemulsion more permeable to the analyte than the micelles. Following this principle, the use of 2-butanol has also been used in EKC with SC-CNTs to increase the permeability of the SC-CNTs [15]. The positive effect of this organic modifier in the peak shape can favour the enantiomeric separation; 2-butanol (0.8, 1.6 and 3.2% v/v) and ethanol (1, 3 and 6% v/v) were thus evaluated. Figure 2A shows the results of $R_s$ obtained for mixtures of (+)- and (–)-norephedrine. As can be seen, baseline separation ($R_s$ value near 1.5) was obtained for 0.8% of 2-butanol and for all the studied percentages of ethanol. Nevertheless, 2-butanol was selected as the organic modifier because it provides a better stability of the baseline. In addition, Table 1 gives the comparison of the $R_s$ values in the presence and absence of 2-butanol in the BGE. Those results show that 2-butanol provides a better enantiomeric separation.

Finally, the influence of the applied voltage in the enantiomeric separation was also studied. Figure 2B shows the values of $R_s$ for norephedrine enantiomers at 10, 15 and 20 kV. As can be seen, the resolution was satisfactory when the applied voltage was lower than 15 kV and it was therefore selected as the optimum taking into account that it resulted in lower migration times, thus reducing the analysis time.

Under the optimum conditions (see Section 2.3), the enantiomeric separation of mixtures of (+)- and (–)-norephedrine, (+)- and (–)-ephedrine and (+)- and (–)-N-methylamphetamine was studied. Figure 3A shows the electropherogram obtained for a standard solution of (+)- and (–)-norephedrine and (+)- and (–)-N-methylamphetamine (individual concentration 5 mg/L). As can be seen, the enantiomers of both analytes were satisfactorily separated using SC-MWNTs as chiral selectors. The resolution factors for norephedrine and ephedrine in the mixture were 1.49 ($\zeta_{enant} = 1.04$) and 1.35 ($\zeta_{enant} = 1.03$), respectively.

Finally, the enantiomers of N-methylamphetamine were also analysed using the optimized conditions for norephedrine enantiomers. As expected, the interaction between the SC-MWNTs was similar to that obtained for ephedrine and norephedrine. An increase of 4 min was observed on the migration time. In addition, the enantiomers of N-methylamphetamine were also separated with the proposed procedure. For these compounds, the values of the resolution and enantioselectivity were 1.48 and 1.03, respectively.

Figure 3B illustrates the electropherogram obtained for a standard solution of (+)- and (–)-norephedrine, (+)- and
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CE and CEC

Figure 2. Resolution values for norephedrine enantiomers using SC-MWNTs 3.2 mg/L and partial filling (6.4 mg/L SC-MWNTs) as a function of (A) percentage of the organic modifier. Experimental conditions: 20 mM boric acid, 20 mM SDS and 15% v/v ACN adjusted at pH 9, voltage 15 kV and an injection time of 10 s. (B) Voltage value, experimental conditions: 20 mM boric acid, 20 mM SDS, 15% v/v ACN and 0.8% 2-butanol adjusted at pH 9 and injection time 10 s.

Figure 3. Electropherogram obtained for a standard solution: (A) 1, (−)-norephedrine; 2, (+)-norephedrine; 3, (−)-ephrine; and 4, (+)-ephrine and (B) 1, (−)-norephedrine; 2, (+)-norephedrine; 3, (−)-ephrine; 4, (+)-ephrine; 5, (−)-N-methylephedrine; and 6, (+)-N-methylephedrine with SC-MWNTs as chiral selector. All the analytes were present at a concentration of 5 mg/L. As can be seen in Fig. 3B, the enantiomers (+)- and (−)-ephrine, (−)-ephrine and (+)-N-methylephedrine were completely resolved under the proposed conditions. Nevertheless, (+)-ephrine and (−)-N-methylephedrine were overlapped and, as stated below, the variation of experimental condition led to the loss of enantioselectivity for one or more enantiomers. Therefore, these two enantiomers could not be properly identified in the case when they were simultaneously present in the same sample.

Finally, an estimation of some analytical parameters was also carried out and they are given in Table 2. Detection limits were between 0.2–0.45 mg/L with linear ranges over the interval 1–25 mg/L. Precision was studied at three concentrations of the linear range, being between 2.7 and 3.2%. A recovery study of the standard solutions was also carried out at two concentration levels and the results were acceptable in all instances with average values of ca. 100%. It should be pointed out that the enantiomeric resolution and enantioselectivity did not depend on the concentration. The sensitivity and precision obtained make the method suitable for the determination of these compounds in pharmaceutical products or illicit drugs.

4 Contributions

Nowadays, different applications of CNTs have been described in the field of Analytical Chemistry. Their particular properties make them especially attractive to be used as filters and membranes, sorbent phases, components of electrochemical biosensors and as separation elements in GC and CE. Those approaches are based on the adsorbent of CNTs or electric properties. Chirality is another important characteristic of...
carbon nanotubes. Physical studies related with optical properties of nanotubes reveal the existence of chirality in those structures [23]. This is a very interesting property for the development of novel analytical applications; indeed, separation of the chiral molecules is one of the most relevant application fields of ECK [10]. Therefore, this paper opens up a promising way of using SC-MWNTs as pseudostationary phase in EKC for developing new direct enantiomeric methods.

5 Concluding remarks and future trends

In the present paper, the potential of SC-MWNTs as chiral selectors for direct enantiomeric methods has been demonstrated. From our point of view, it is a significant advance in nanotubes applications because it allows the separation of enantiomers, in a rapid and simple way, being a very interesting alternative to other chiral selectors like CDs or MEECK based chiral methods.

The combination of MWNTs' sonication (for inducing chirality) and the optimum composition of the electrophoretic buffer together with the use of partial filling leads to the development of an efficient enantiomeric separation method. Notwithstanding this, electrophoretic separation of (+)-ephrine and (-)-N-methyl ephephrine enantiomers was not possible, which collapsed as a result of the resolution of the ephrine and N-methyl ephrine enantiomers.

SWNTs were also tested but the results obtained did not permit the development of chiral separation of the target analytes as the experimental conditions did not improve chiral interaction between the analytes and the nanotube surface.

Additional studies will be required to elucidate the chiral interactions between the analytes and the surface of MWNTs and for the development of new and robust methodologies.

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6 References
