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Short communication

Evaluation of carbon nanotubes as chiral selectors for continuous-flow enantiomeric separation of carvedilol with fluorescent detection☆

Raúl A. Silva[a], Maria C. Talío[c], Marta O. Luconi[b], Liliana P. Fernández[a,b,c,*]

[a] Área de Farmacotecnia, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Argentina
[b] Área de Química Analítica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Argentina
[c] INQUISAL-CONICET, Chacabuco y Pedernera, 5700 - San Luis, Argentina

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ABSTRACT

Single-walled carbon nanotubes (SWNT) are proposed as chiral selectors for separation of carvedilol stereoisomers beginning since its racemic mixture. The novel developed FIA-methodology employs a microcolumn (mC) packed with a few milligrams of SWNT which showed to be effective in S(−) and R(+) carvedilol separation. Attending to spectral properties of analytes, molecular fluorescence was employed in the detection step. Separation of carvedilol enantiomers was achieved in less than 70 s with an acceptable resolution factor of 3.16. Variables that influence the chiral separation such as pH and composition of eluent solution, sample injection volume and flow rate, activation mode of NTs and mass of the same in column have been examined in detail. At optimal operational conditions, well repeatability was achieved using the same column for more than 100 injections, putting in evidence the stability of nanomaterial and the efficacy and versatility of the proposed FIA-configuration. The new methodology was successfully applied to S(−) and R(+) carvedilol quantification in pharmaceutical preparations, resulting an attractive alternative to traditional separative methods being fast, simple, using low cost instrumentation and producing scarce waste.

1. Introduction

The separation of chiral compounds is of great importance in several industries. In pharmaceuticals area, different enantiomeric forms are known to have different physiological and therapeutic effects. Methods currently available for determining of enantiomeric purity are based on either separations [1–4] or spectroscopic methodologies [5]. Although these methods in the main cases have proven to be effective, they all have some drawbacks, such as time-consuming or destructive and low sensitivity [6].

Although there are many sensitive and selective analytical methodologies, sample treatment continues being a fundamental step in achieving quality results. CNTs have been the subject of intense research because of their extraordinary physical, chemical, and electrical properties [7,8]. They also provide unique opportunities for the development of high performance separation techniques that utilize nanoscale interactions in a material [9–11]. Carbon nanotubes have been also used as adsorbents in solid phase extraction (SPE) for preconcentration and traces determinations in environmental samples [12]. Recently, results have been reviewed as strategy for improving analytical problems [13]. Surface modification and functionalization of CNT's proved itself as a powerful tool for improving the selectivity and efficiency of the SPE [14].

Monolithic organic polymer stationary phase containing SWNTs has been used in HPLC and capillary electrochromatography [15,16].

Spectroscopy studies of SWNTs have demonstrated the existence of three configurations, attending the direction of graphene sheet: two non-chiral structures (armchair and zig-zag) and, the other denominated chiral [17]. Experimental assays have shown that interaction between chiral compounds and the chiral fraction of SWNTs can exit.

Carvedilol is a non-selective β-blocker indicated in the treatment of mild to moderate congestive heart failure. It also has vasodilating properties that are mainly attributed to its α1-blocking activity, as well as ability to inhibit oxidative stress in coronary smooth muscle [18].

Carvedilol is administered as a racemic mixture of the R(+) and the S(−) enantiomers (Fig. 1). Enantiomers exhibit different pharmacological effects; the β-receptor blocking activity of the S(−) enantiomer is about 200-fold higher than that of R(+) carvedilol,

☆ "In memoriam" of Dr. Adriana Masi (1962–2010), prominent researcher, dear colleague and friend, who passed away prematurely, as a consequence of public insecurity, killed by a shot in the head at the door of her house.

* Corresponding author at: Área de Química Analítica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina.
Tel.: +54 2664 430224; fax: +54 2664 430224.
E-mail address: lfernand@unsl.edu.ar (L.P. Fernández).

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whereas both enantiomers are equipotent α-blockers [19]. In order to get information about pharmacokinetic–pharmacodynamic relations, it is therefore necessary to measure individual carvedilol enantiomer concentrations.

In this work, SWNT is proposed as chiral selectors for separation of carvedilol racemic mixture using a FIA-configuration with fluorescence detection.

2. Materials and methods

2.1. Instrumental

A Shimadzu RF-5301PC spectrofluorimeter (Shimadzu Corporation, Analytical Instrument Division, Kyoto, Japan), equipped with a Xenon discharge lamp was used. For the fluorescent measurements, quartz cells of 1 cm were used in statically assays and for flow measurements a LC flow-cell unit of 12 μL was used.

A Rheodhyne low pressure valve Model 5020 (Rohnert Park, CA, USA) with a fixed volume loop injector of 2.8 mL was used for FIA configuration.

Solutions were propelled by Gilson Minipuls 3 peristaltic pumps (Middleton, WI, USA) with PVC pumping tubes. All tubing connecting the different components of the flow system was PVC, 0.8 mm i.d.

A pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) Model EA 940 with combined glass electrode was used for monitoring pH adjustment.

2.2. Reagents

Carvedilol was kindly provided by Gador S.A. (Buenos Aires, Argentina). Sodium dodecylsulfate (SDS), Triton® X-100 and hexadecyl trimethylammonium bromide (HTAB) were purchased from Tokyo Kasei Industries. Chuo-Ku, Tokyo, Japan. SWNT were purchased from Merck (Darmstadt, Germany).

The pH values in optimization stage were adjusted by the addition of solutions of NaOH 0.01 mol L^{-1}, NaOH (c), HCl 0.01 mol L^{-1} or HCl (c) until the target pH value was reached.

All used reagents and solvents were of analytical or spectroscopic grade.

2.3. Solutions

Carvedilol standard solution containing 1 mg mL^{-1} was prepared dissolving the reagent in ethanol. This solution was found to be stable for several weeks when it was kept in dark at 5 °C. Standard working solution of 1 μg mL^{-1} was prepared daily by dilution of standard solution with doubly distilled water and stored in a dark bottle at 5 °C. In these conditions, carvedilol was stable for almost four weeks.

A 0.1 mol L^{-1} SDS solution was prepared with an adequate weight of SDS and dissolving in doubly distilled water.

A 0.1 mol L^{-1} HCl solution was prepared mixing an adequate volume of concentrated acid with doubly distilled water.

2.4. SWNTs activation

Several small portions of SWNTs were put into Erlenmeyers and washed each with different solutions: H_2SO_4, HCl, HNO_3 and NaOH, in all cases 2 mequiv. mL^{-1}. Moreover, a mix of H_2SO_4:HNO_3 (50:50) was too assayed in activation step. Then, SWNTs were raised with doubly distilled water for removing the excess of used reactive, filtered throw filter paper (S&S blue band) and dried at ambient temperature. NTs were reserved in desiccator at the moment of preparing the mC.

2.5. Sample preparation

Ten tablets of Bidecar (Baliarda Laboratory, Argentine) containing 12.5 mg of carvedilol each were finely powdered. A portion of the powder equivalent to 10 mg of carvedilol was weighed and treated with doubly distilled water, shaken for 15 min and centrifuged for 30 min, in order to remove soluble substances. Supernatant was eliminated and the residue was treated with 10 mL of absolute ethanol. The solution was filtrated through membrane disc of 0.45 μm pore size. Successive dilutions were realized until a final carvedilol concentration equivalent at 0.1 μg mL^{-1} and reserved for the application of developed methodology.

2.6. FIA configuration. General procedure

Fig. 2 shows a schematic representation of the FIA system used for the separation of carvedilol racemic mixture with spectrofluorimetric detection. The manifold used was built using a peristaltic pump with rate selector; an injection valve acting as selecting valve, and PVC tubing of 0.8 mm i.d.

A stream of HCl/SDS (eluent in Fig. 2**a**) was propelled as reagent blank, for producing baseline. After changing the valve position (Fig. 2**b**), a stream of carvedilol standard solution or sample (2.8 mL) was impelled through the SWNT mC and conducted to fluorimetric detector (λ_{exc} = 317 nm; λ_{em} = 358 nm) generating enantiomeric signals.

3. Results and discussion

3.1. Activation of SWNTs

In order to optimize SWNTs adsorptive properties, various kings of chemical reagents and mixtures were used as surface modifies. Among assayed reagents, HNO_3 (2 mequiv. mL^{-1}) showed to be the best for activating SWNTs; so, nanomaterials treated with HNO_3 presented the best performance in carvedilol enantiomeric separation. Shim et al. have justified this behavior saying that oxidation process improves nanomaterial dispersivity and increases a large

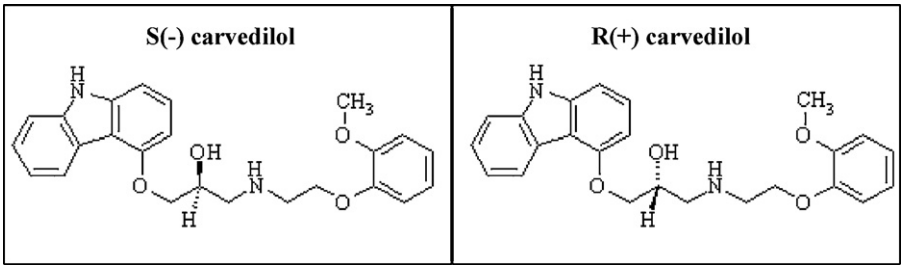


Fig. 1. Chemical structure of carvedilol stereoisomers.

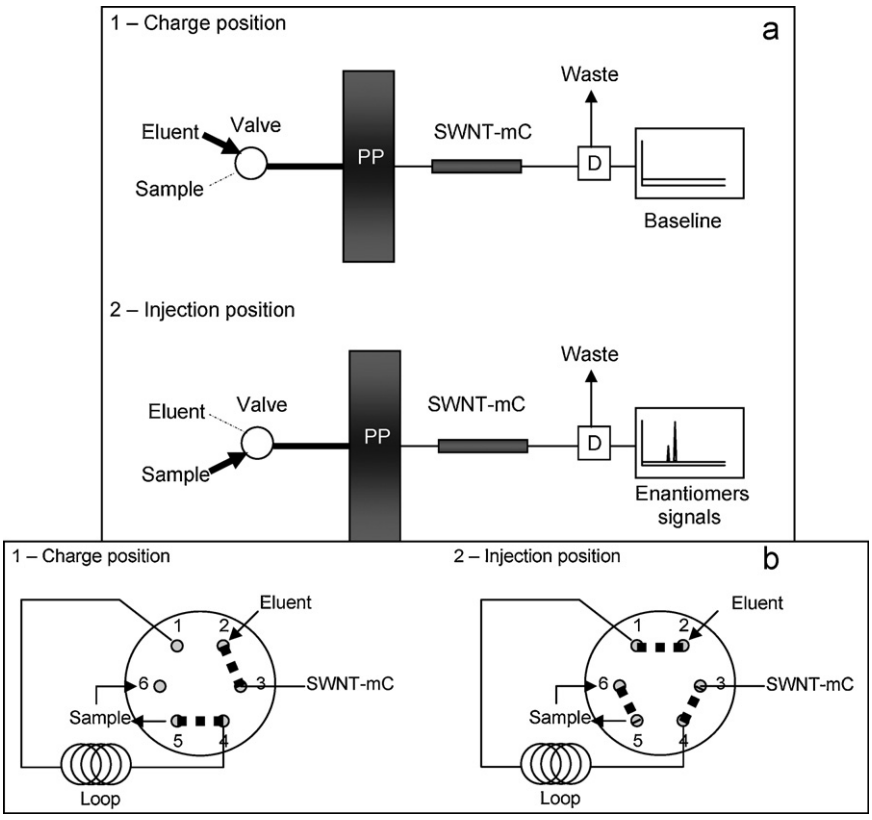


Fig. 2. (a) Schematic representation of the FIA system for the separation of carvedilol racemic mixture with spectrofluorimetric detection. (b) Valve detail.

amount of oxygen-containing functional groups like –COOH, –OH, or –CO on the surface site of CNTs [20].

These nanomaterials were chosen as packed material of the mC in FIA system.

3.2. Arranging the mC for FIA system

A glass tube of 1.5 mm i.d. was chosen for containing the SWNTs. Several materials were assayed for retaining nanomaterials into the column. Cotton and filter paper underwent severe compaction when solutions flowed throw the column, causing uncouples in FIA system. Wool glass resulted adequate not only in SWNTs retention into the mC, but also permitting the free pass of reagents flow in FIA system. Otherwise, a stable baseline was obtained for this column in fluorescence detector.

Other important parameter studied was the optimal mass of nanomaterial that guarantees the analyte separation. Mini column was packed with 1.2, 2.5 and 3.6 mg of SWNTs. In all cases, no variation in carvedilol racemic resolution was observed. Also, 1.2 mg of nanomaterial as economical option was chosen for the packing mC.

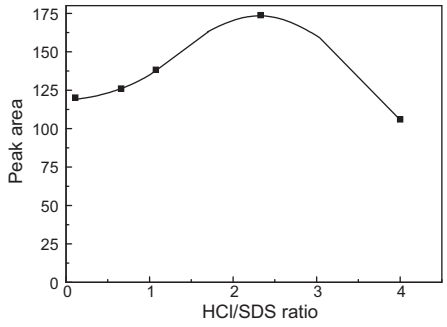
3.3. Choosing the eluent

Experiments were carried out in order to explore the optimal eluent that show a good carvedilol isomers resolution. Taking into account that surfactants have produced a beneficial effect in racemic mixture separations [21], solutions were preparing using HCl/SDS mixtures at different ratios. Fig. 3 shows the obtain results using R(+) carvedilol peak areas employing HCl/SDS mixtures in ratios from 0 to 4. The best peak area was obtained for a ratio of 2 for HCl (0.1 mol L^{-1})/SDS (0.02 mol L^{-1}) mixture and was chosen for following assays. Similar results were obtain using S(−) peak area data.

3.4. Carvedilol spectral studies in micellar medium

Molecular fluorescence spectroscopy constitutes a group of instrumental methodologies characterized by high sensitivity, adequate selectivity and ample lineal range. These advantages show that they are very attractive as detectors in FIA configurations.

Carvedilol presents a strong native fluorescence when is irradiated with light of UV–vis range. In previous studies can be established that SDS micellar medium produces an enhancement in racemate carvedilol fluorescent signal [22]. Carvedilol showed a maximum emission at 358 nm in micellar medium when was excited at 317 nm. These wavelengths were selected for the following assays to measure the fluorescence intensity.



$C_{Carvedilol}$ = 0.1 μg mL^{-1}; C_{HCl} = 0.1 mol L^{-1}; C_{SDS} = 0.02 mol L^{-1}; Flow rate = 4.2 mL min^{-1}; Volume injection = 2.8 mL; λ_{exc} = 317 nm; λ_{em} = 358 nm; Excitation slit/ Emission slit = 3/5.

Fig. 3. Influence of eluent composition on R(+) carvedilol peak area.

Table 1
Analytical parameters of developed methodology.

Parameter	S(−) carvedilol	R(+) carvedilol
Linearity range	9.4×10^{-6}–0.1 μg mL^{-1}	5.6×10^{-7}–0.1 μg mL^{-1}
LOD	2.8×10^{-6} μg mL^{-1}	1.7×10^{-7} μg mL^{-1}
LOQ	9.4×10^{-6} μg mL^{-1}	5.6×10^{-7} μg mL^{-1}
Calibration sensitivity	4885.1 mL μg^{-1}	82.234 mL μg^{-1}
Correlation coefficient (r^2)	0.996	0.997
SD	0.236	1.156

3.5. Optimization of FIA parameters

The effect of flow rate of FIA configuration on carvedilol stereoisomers separation was studied; a good response was obtained using 4.2 mL min^{-1}. For higher flow rate, turbulences were observed due to the introduction of bubbles into the FIA system. So, a flow 4.2 mL min^{-1} was selected as optimal. The proposed FIA configuration permitted to realize injections of sample every 40 s; a sampling rate of 90 samples h^{-1} was attained.

Assays were carried out in order to establish the optimal volume of sample injection compatible with column capacity allowing an adequate resolution of carvedilol isomers. Likewise, volume of carvedilol must be selected taking into account the instrumental sensitivity at working conditions. An analyte injection of 2.8 mL showed good separation performance and it was selected for following assays.

4. Analytical performance and validation

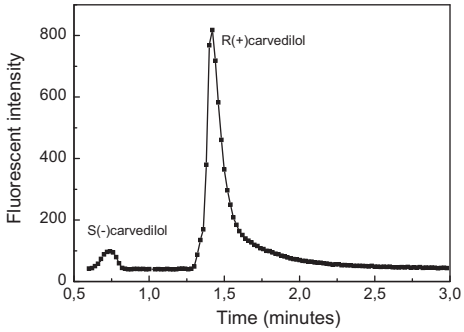
The proposed methodology was validated in terms of linearity and precision. The precision of the method based on repeatability was performed, by replicating injections ($n = 6$) of three standard solutions covering different concentration levels.

By using the above procedure, a linear regression equation was obtained. The regression plots showed that there was a linear dependence of the peak area on the concentration of the drug over the ranges cited in Table 1.

The limit of detection (LOD) was calculated as $3s/m$, where s is the standard deviation of 10 successive means of the blank and m is the slope of the calibration curve (calibration sensitivity). The limit of quantification (LOQ) was calculated as $10s/m$. Range of linearity was evaluated by checking the linear regression coefficient (r^2) of the calibration curve [23]. The proposed methods were evaluated for the precision as standard deviation (SD).

The obtained results have permitted to arrive to important conclusions with respect to the different behavior of carvedilol stereoisomers in this separative process:

– A very short retention time of 23 s was obtained for S(−) isomer, putting in evidence a poor affinity of this isomer for SWNTs. Obtained sensitivity resulted as that informed by carvedilol aqueous solution [20].



Flow rate = 4.2 mL min^{-1}; Volume injection = 2.8 mL; λ_{exc} = 317 nm; λ_{em} = 358 nm; Excitation slit/ Emission slit = 3/5.

Fig. 4. Fiagram signals of pharmaceutical sample of racemic carvedilol.

– R(+) isomer was concentrated remaining into mC until HCl/SDS mixture was used as eluent, showing high affinity for nanomaterial. Improved signals were achieved due to additive effect of preconcentration and micellar enhancement.

This fact constitutes an important experimental advantage in relation to the possibility of regulating the resolution factor [24] in isomeric separation.

In order to explore the purity of each isomer separated, fractions of S(−) carvedilol and R(+) carvedilol were collected in separated recipes and re-injected to FIA system. The fiagram for each isomer showed here consists of only one peak, putting in evidence the perfect purity of each carvedilol enantiomer obtained from separation proposed methodology. Additionally, the optical rotation for each isolate isomer was in concordance with published data [25].

5. Application

The developed method was applied to the separation of carvedilol isomers in a commercial pharmaceutical containing 12.5 mg of racemic mixture. Fiagram for sample injection is represented in Fig. 4.

No interference was observed in fiagram due to the presence of excipients. However, after ten injections of samples, it was necessary to implement a recycled step to mC, washing it with eluent solution during one minute. This strategy produced the elution of all matrix compounds retained in SWNTs and prepared the mC for following assays, without producing alteration in its analytical performance.

Table 2 shows the results for quantification of S(−) and R(+) carvedilol in a pharmaceutical formula commercialized in Argentine. Considering the satisfactory recuperations obtained, the new methodology represents a good quality alternative to standard separative methods.

Table 2
Recovery study. Determination of carvedilol stereoisomers using the developed methodology.

Sample	Added (μg mL^{-1})		Found ± s (μg mL^{-1})	Recovery (%, $n = 6$)
Bidecar tablets	S(−) carvedilol	–	0.068 ± 0.03	–
		0.02	0.086 ± 0.02	97.06
		0.04	0.109 ± 0.04	101.50
		0.06	0.128 ± 0.02	100.00
	R(+) carvedilol	–	0.045 ± 0.04	–
		0.02	0.064 ± 0.05	97.80
		0.04	0.084 ± 0.02	97.80
		0.06	0.107 ± 0.05	104.44

6. Conclusions

A minicolumn filled with SWNT has been employed for separation of carvedilol racemic mixture using a FIA-configuration with fluorescence detection. Nanomaterials were activated with different substances, resulting HNO_3 (2 equiv. L^{-1}) the most adequate for analyte retention, producing oxidation process that improves isomeric separation. FIA parameters were optimized; using a flow rate of 4.2 mL min^{-1} satisfactory separation of R(+) and S(−) carvedilol was obtained with a resolution factor of 3.16 and a sampling rate of 90 samples h^{-1}. S(−)carvedilol showed a retention time of 23 s when was eluted with water; R(+) carvedilol presented a retention time of 65 s using HCl/SDS mixture. An important advantage, worthy of being highlighted, is related with the well stability and repeatability achieved using the same column during more of 100 injections, putting in evidence the stability of nanomaterial and the efficacy and versatility of the proposed FIA-configuration. The proposed methodology is characterized by adequate selectivity and sensitivity, constituting an alternative simple and inexpensive to the available methods for the determination of carvedilol isomers, does not being necessary time-consuming derivatization steps.

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