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Chiral separation and quantitative analysis of citalopram by modified capillary electrophoresis

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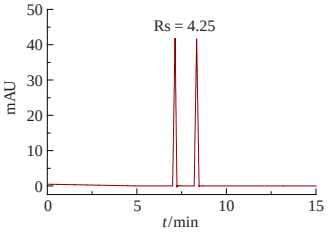
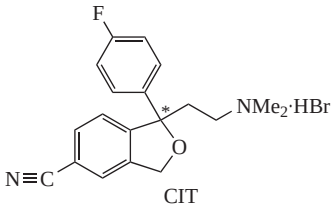
A novel capillary for the high efficiency splitting of citalopram based on modified nanosize silica was designed and synthesized. The enantiomers of citalopram were separated within 10 min with a resolution (Rs) of 4.25.



Capillary electrophoresis is widely used in the analysis of chiral drugs.[1–8] High performance liquid chromatography (HPLC) is a traditional chiral separation method, which has high load capacity and high solvent consumption. Capillary columns developed for HPLC require a highly sensitive detector,[9–11] *e.g.*, in a combination of liquid chromatography (LC) with mass spectrometry, however this instrument is expensive. In recent years, chiral supercritical fluid chromatography (SFC) has emerged as a technique for analytical, semi-preparative and preparative separation of enantiomers in the pharmaceutical industry due to advantages in speed, high column efficiency and significantly lower mobile-phase consumption than conventional LC.[12] Counter-current chromatography is a new efficient chromatographic technique based on continuous liquid–liquid partition. Recently, it attracted more attention in chiral separation due to its high load capacity, cheap liquid stationary phase and low solvent consumption.[13] Enantiomeric separation is a critical subject in pharmaceutical analysis because the enantiomers of a racemic drug usually display different pharmacological activities. For this reason, rapid, selective and effective analytical methods are required to verify the enantiomeric purity of chiral drugs. Recently, capillary electrophoresis (CE) has been used in chiral separation in view of its simplicity, high efficiency and low cost. A variety of compounds including native β-cyclodextrins (β-CDs) and their derivatives, polysaccharides, antibiotics, polymeric surfactants and ionic liquids (ILs) have been effectively used as chiral selectors in CE.[14] The most commonly used chiral selectors are CDs due to their wide variety of cavity size, side chain, degree of substitution (DS) and charge. Small changes in the CD structure, substitution pattern or cavity size could lead to different affinity toward analyte isomers.[15–17] CE has many operation modes including electrokinetic chromatography, micellar electrokinetic chromatography, microemulsion electrokinetic chromatography and capillary electrochromatography.

Citalopram hydrobromide [CIT, *S*-(+) and *R*-(–)], a selective serotonin reuptake inhibitor developed by Lundbeck, is suitable for the acute and regular treatment of depressive disorder.[18] It has a high therapeutic index, and a low incidence of adverse reactions.[19] However, its overtime application can damage the liver, imbalance body chemicals, and eventually cause depression anxiety. Pharmacological studies[20] showed that the *S*-(+)-CIT selective inhibitory effect is stronger, the metabolites *S*-(+)-DM-CIT and *S*-(+)-DDM-CIT also have a certain effect, whereas *R*-(–)-CIT and its metabolites have a little effect.



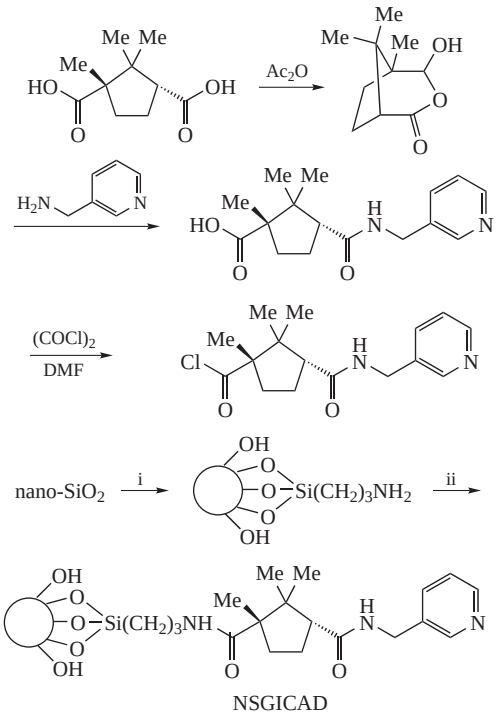
CIT

Therefore, the chiral separation analysis of CIT is of great significance, to control the quality of pharmaceutical ingredients and pharmacodynamics research. The chiral separation of CIT[21] by a chiral column has been reported, however, that chiral column was expensive. The capillary electrophoresis chiral separation of CIT with a sulfonated β-cyclodextrin chiral selection agent was described.[22,23] Our study was aimed at finding a cheap analysis method to perform CIT chiral separation of enantiomers and the determination of the *S*-(+)-CIT isomer.

Herein, we studied the main factors affecting the chiral separation of *S*-(+)-CIT and *R*-(–)-CIT by CE using a modified capillary.†

The procedures for preparation of the D(+)-camphoric acid-derivatized capillary are illustrated in Scheme 1 (for details, see Online Supplementary Materials). Prior to modification, the silica

† Separations were performed on a P/ACE 2100 apparatus (Beckman Instruments) equipped with a diode array detector (DAD). A fused silica capillary (Polymicro Technology) of 75 μm i.d. (375 μm o.d.) and 57 cm in total length (50 cm to the detector) was used. Samples were injected in the hydrodynamic mode by overpressure (3.43×10^3 Pa). The System Gold software was used for data acquisition. All experiments were carried out at 25 °C using a liquid thermostated capillary cartridge except the temperature experiment. LC-10Atvp HPLC was from Shimadzu Co., PHS-3C acidometer from Shanghai Leici analysis instrument factory.



NSGICAD

Scheme 1 *Reagents and conditions*: i, $(EtO)_3Si(CH_2)_3NH_2$, MeOH, 55 °C, ~18 h; ii, (1*R*,3*S*)-2-chlorocarbonyl-1,2,2-trimethyl-3-[*N*-(3-pyridylmethyl)-carbamoyl]cyclopentane pretreated with MeOH and AcOH at 90 °C, 60 °C, ~18 h.

capillary was rinsed successively with 1.0 M NaOH for 20 min, water for 20 min, 1.0 M HCl for 1 h, water for 20 min and then dried in a gas chromatography (GC) oven at 110 °C for 10 h. Next, 0.50 g of nanosize silica was dispersed in 5.0 ml of 0.01 M acetic acid solution. The obtained liquid disproportion was loaded into the above capillary by injection. Thereafter, the capillary was attached to HPLC; HPLC-grade acetonitrile was passed for 3 h to remove dispersant. The capillary was placed in a GC oven and kept at 300 °C for 12 h to remove acetonitrile. The obtained capillary was rinsed with 0.10 M NaOH solution for 1 h, and then with water to pH 7.0. After subsequent flushing with methanol for 10 min, it was purged by nitrogen gas for 30 min (see Figure S1, Online Supplementary Materials).

Modification of nano-SiO_2 in the capillary was performed as follows. First, the solution of APES (0.10 mmol) in methanol (1:1, v/v) was syringed into the capillary, and it was kept at 55 °C overnight after both ends sealed with rubber in GC oven. Then, the capillary was rinsed with methanol and deionized water successively to flush out the residual reagents and dried again. This filling of the capillary was finally derivatized with chiral camphor acid monoamide monochloride to obtain the target NSGICAD (see Scheme 1).

The reference material (C-NSGICAD) was prepared using the same procedure (see Scheme 1) in a beaker and was studied by IR, TGA and elemental analysis. The IR spectra of free nanosize silica exhibit peaks at 3438.6, 1642.1 and 1089.8 cm^{-1}, while those of C-NSGICAD, at 3437.2, 2979.2, 2951.6, 1783.1, 1701.0, 1685.6, 1462.0, 1320.2, 1283.8, 1243.8, 1089.0 and 943.4 cm^{-1}. The new adsorption peaks of C-NSGICAD can be attributed to (1*R*,3*S*)-camphoric acid and pyridine. This provides an evidence for the attachment of (1*R*,3*S*)-camphoric moiety onto the nanosize silica surface.

During TGA analysis, C-NSGICAD started to lose water molecules as the temperature was raised to 100 °C, and then the curve kept slow decline until a sharp decrease occurred at 200 °C. Compared to free nanosize silica, the added decrease of C-NSGICAD can be ascribed to the loss of an organic block containing (1*R*,3*S*)-camphoric acid-derivative group, accounting for about 25.1%. The TGA of free nano-SiO_2 looks differently (see Figure S2, Online Supplementary Materials).

To evaluate the amount of (1*R*,3*S*)-camphoric acid loaded to the surface of nanosize silica, elemental analysis was performed. The mass percentage of carbon, hydrogen and nitrogen of C-NSGICAD were found to be 26.65, 3.81, and 5.56%. The value of 5.56% nitrogen corresponded to a 4.31 μmol m^{-2} surface density of organic component, which was lower than 4.73 μmol m^{-2} of the surface single molecular distribution density of amine group. This suggested that the reactions between $(EtO)_3Si(CH_2)_3NH_2$ and nanosize silica and/or amine group with (1*R*,3*S*)-camphoric acid derivative were not complete.

Since the resolution and migration time in CE was affected by separation voltage, the influences of the citalopram separation voltage on resolution and migration time in CE were examined in a range of 10 to 30 kV. It was found that citalopram resolution increased gradually and the migration time became shorter as the separation voltage increased. Thus, a separation voltage of 30 kV was selected.

The influence of citalopram detection wavelength on detection signal was examined at the wavelengths of 210.0, 220.0, 230.0, 240.0 and 250.0 nm. The highest detection sensitivity was observed at 240.0 nm.

The electrophoretic results of CE on NSGICAD capillary in different buffers such as hexamine–HCl (15.0 mmol dm^{-3}, pH 5.40), CAPS (15.0 mmol dm^{-3}, pH 8.70) and phosphate (15.0 mmol dm^{-3}, pH 9.10) (see Figure S3, Online Supplementary Materials) have shown that the CAPS buffer and the phosphate buffer have low resolution, while hexamine–HCl buffer provided high resolution and peak shapes were good. Thus, the hexamine–HCl buffer was selected for the following experiments.

The resolution turned to be dependent on running buffer concentration and pH value (see Figure S4, Online Supplementary Materials). The resolution increased rapidly as the pH value was raised from 3.0 to 5.40 and then it decreased. Thus, pH 5.40 was selected. Also, the resolution slowly diminished as the buffer concentration increased from 5.0 to 25.0 mmol dm^{-3} and then it dropped rapidly when the buffer concentration was above 25.0 mmol dm^{-3}. Since the migration time grew as the buffer concentration decreased, a hexamine–HCl buffer concentration of 15.0 mmol dm^{-3} was selected to shorten the time of measurement with a relatively higher resolution.

Migration time and resolution was also affected by analysis temperature. They decreased as the temperature was increased from 10 to 30 °C; so temperature of 25 °C was selected (see Figure S5, Online Supplementary Materials).

Under the optimum experimental conditions, the electropherogram looked as outlined in Figure 1. The resolution (Rs) of citalopram was 4.25.

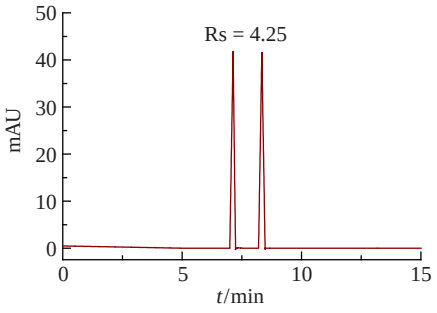


Figure 1 The splitting of citalopram under optimal conditions: voltage, 30 kV; temperature, 25 °C; sample injection pressure, 3.43×10^3 Pa; duration, 10 s; citalopram, 0.10 mmol dm^{-3}; detection wavelength, 240.0 nm; and pH 5.40.

Under the optimal conditions, the CE intensity has a linear relationship with the concentration of citalopram in a range of 0.03–0.20 mmol dm^{-3}; the linear regression equation is $y = 0.612x - 15.2$, $r = 0.9988$. After the 13-times determination of 0.035 mmol dm^{-3} citalopram, the peak high RSD is 0.22%, the migration time RSD is 0.45%. The detection limit (3σ) is 0.022 mmol dm^{-3}. The measurements were well reproducible (see Tables S1 and S2, Online Supplementary Materials).

In conclusion, a novel capillary of high efficiency splitting citalopram based on modified nanosize silica was designed and synthesized. The modified capillary was applied to capillary electrophoresis with a diode array detector (DAD), which was used for the separation splitting and determination of citalopram. The experimental conditions were optimized: running buffer, pH 5.40; 15.0 mM hexamine–HCl buffer; separation voltage, 30 kV; and temperature, 25 °C. The sample solution was injected for 10 s in the hydrodynamic mode (3.43×10^3 Pa). The linearity range was 0.03–0.20 mmol dm^{-3}, the detection limit was 0.022 mmol dm^{-3}, and the relative standard deviation was lower than 0.45%. The resolution (Rs) of citalopram was 4.25.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2016.03.029.

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