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Research Article

The first contribution of capillary electrophoresis to the study of abiotic origins of homochirality: Investigation of the enantioselective adsorption of 3-carboxy adipic acid on minerals

CE with UV detection was used for the first time to determine the enantioselective adsorption of the short-chain tricarboxylic acid, 3-carboxy adipic acid, on minerals as a mean of investigating plausible mechanisms for the origin of biochemical homochirality on Earth. The use of vancomycine as chiral selector in the separation buffer using the partial filling technique enabled the separation of the two enantiomers of this organic acid in about 12 min. Taking into account that this compound has a low absorption of the UV light, and in order to achieve the sensitivity needed to determine the enantiomeric excess of samples of 3-carboxy adipic acid adsorbed on minerals, we applied a strategy consisting of a field-amplified sample stacking together with the use of a bubble capillary and detection at low wavelength (192 nm). This combination enabled an LOD of about 10^{-7} M and the determination of the enantiomeric excess of 3-carboxy adipic acid adsorbed on calcite and feldspar mineral samples at subnanomol levels of this acid. Results showed that an enantioselective adsorption of the enantiomers of 3-carboxy adipic acid on minerals took place.

Keywords:
Capillary electrophoresis / Homochirality / Minerals / Short-chain tricarboxylic acid
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1 Introduction

A key question related to the origin of life is the emergence of biological homochirality, that is, the almost exclusive one-handedness of chiral molecules in living organisms (*i.e.*, L-amino acids and D-sugars) [1]. Several theories have been proposed to explain the origins of homochirality [2]. Some of these theories state that life was dependent on achiral molecules and racemates, and that homochirality arose through subsequent biological evolution. Other theories consider that homochirality was a prerequisite for the emergence of life, and that enantiomerically enriched asymmetric molecules were produced and/or delivered before living organisms existed. In that regard, some authors claim that enantiomerically enriched amino acids formed in interstellar nebulae or the prebiotic solar system, and then were delivered to Earth by meteoritic carbonaceous chondrites [1]. Their assumptions were supported by the GC-MS determination of enantiomeric excesses (e.e.) of some amino acids extracted from meteorites, finding certain preponderance of the L-counterpart. It was postulated that enantiomeric enrichment of these organic compounds resulted from photochemical degradation due to exposure of meteoritic material to UV circularly polarized light and then delivered to Earth. Other plausible scenarios to explain the origins of homochirality have also been proposed. Asymmetric crystalline mineral surfaces, which provide effective environments for chiral molecular discrimination and accumulation in a natural context, have been cited for almost 70 years in reference to their possible role in the origins of biochemical homochirality [3–6]. Accordingly, Hazen *et al.* [7] demonstrated that calcite crystals, when immersed in a racemic solution of aspartic acid, showed significant adsorption and chiral selectivity of the D- and L-enantiomers upon mirror-related crystal surfaces.

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Abbreviations: DMA-EPyM, *N,N*-dimethylacrylamide-ethylpyrrolidine methacrylate; **e.e.,** enantiomeric excesses; **FASS**, field-amplified sample stacking; **HDB,** hexadimetrin bromide; **HP-β-CD,** 2-hydroxypropyl-β-CD; **pzc,** point of zero charge; **VC,** vancomycine

These results raise the possibility that other molecules might display enantioselective adsorption on mineral surfaces, such as calcite or alkali feldspar, which are ubiquitous in Earth's crust. Such surfaces provide the most abundant and accesible local chiral geochemical environments, and represent logical sites for the prebiotic chiral selection and organization of essential biomolecules [8]. Thus, following this line, we turned our attention to a chiral tricarboxylic acid, namely 3-carboxy-1,6-hexanedioic acid, or 3-carboxy adipic acid (Fig. 1). We chose this tricarboxylic acid as candidate for adsorption studies for two main reasons. First, 3-carboxy adipic acid bears in its molecule the three noncolinear functional groups necessary for enantioselective adsorption with a solid surface, according to the "Ogston three point attachment" interaction model [9, 10]. Second, 3-carboxyadipic acid has prebiotic relevance as it has been reported to result as a minor product of the γ-radiolysis of succinic acid, which is a well-known product of several reported prebiotic syntheses of organic compounds. For these reasons, it can be considered that 3-carboxy adipic acid could have been formed and accumulated in Archaean Earth [11, 12].

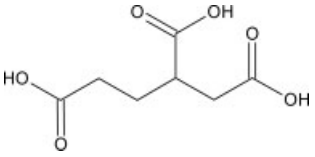

Figure 1. Structure of 3-carboxy adipic acid.

In order to conduct enantioselective adsorption experiments of 3-carboxy adipic acid on mineral surfaces, the availability of an analytical method enabling the separation of both enantiomers to calculate e.e. for 3-carboxy adipic acid was necessary. As no analytical protocols have been reported, CE was chosen in order to investigate the desired separation. With this technique it is simple to construct and modify a chiral environment, which is the key to resolve these complex isomers. However, chiral analysis of nonaromatic short chain organic acids is very complicated, mainly because the steric factors providing the basis for chiral recognition are very limited for these compounds [13]. In addition, these methodologies suffer from the problem of poor sensitivity in UV detection, because short chain organic acids do not contain chromophore group. Relatively recent works have shown the possibility of direct chiral resolution of various aliphatic mono- and dicarboxylic acids by CE using mainly CDs or macrocyclic antibiotics [13]. In the case of CDs, previous results have shown that the most effective CD has been 2-hydroxypropyl-β-CD [14–19], but the use of CDs to resolve the enantiomers of aliphatic mono- and dicarboxylic acids only seems to enable good results when high concentrations (molar level) are used, which is an important drawback due to its high cost. However, macrocyclic antibiotics, such as vancomycine (VC), have shown powerful enantioselectivity toward aliphatic carboxylic acids at low concentrations (millimolar level) [20–23]. In addition, VC is one of the most powerful chiral selectors for the separation of anionic compounds, in particular those containing free carboxylic groups [20–30]. Nevertheless, VC as all macrocyclic antibiotics, exhibits some drawbacks such as the adsorption on the capillary wall due to its cationic charge in the pH range from 4 to 7 [31–33], which results in poor separation efficiency [34], and the strong absorption in the UV wavelengths that causes a decreased detection sensitivity [25]. In spite of the methods reported for the separation of aliphatic mono- and dicarboxylic acids by CE, no results have been reported on the separation of tricarboxylic acids.

In this work, a rapid and sensitive CE-based analytical method enabling the separation of the enantiomers of 3-carboxy adipic acid and without previous derivatization is proposed. This is the first time that the chiral separation of 3-carboxy adipic acid is reported. The application of the developed CE method to the determination of the enantiomeric excess of this tricarboxylic acid in samples adsorbed on minerals is also presented.

2 Materials and methods

2.1 Reagents and samples

All reagents employed for the preparation of the BGE were of analytical grade. Water used to prepare solutions was purified through a Milli-Q system from Millipore (Bedford, MA, USA). Orthophosphoric acid, sodium dihydrogen phosphate dihydrate, sodium hydroxide, hydrochloric acid, formic acid, ammonium hydroxide, and dodecyl sulfate sodium salt were supplied from Merck (Darmstadt, Germany). Methanol (MeOH), isopropanol, chloroform, and ACN (MeCN) were purchased from Scharlau (Barcelona, Spain). 2-Hydroxypropyl-β-CD (HP-β-CD) was from Fluka (Buchs, Switzerland). VC, hexadimetrin bromide (HDB), and racemic 3-carboxy adipic acid were supplied from Sigma (St. Louis, MO, USA). The copolymer *N,N*-dimethylacrylamide-ethylpyrrolidine methacrylate (DMA-EPyM), which synthesis has been described [35], was kindly provided by Dr. Alejandro Cifuentes (Institute of Industrial Fermentations (CSIC), Madrid, Spain).

The enantiomerically enriched (−)-*S*-3-carboxy adipic acid was prepared dissolved the racemic 3-carboxy adipic acid (62 mg, 0.326 mmol) and anhydrous (−)-brucine (310 mg, 0.787 mmol) in absolute ethanol (4 mL) and heated to reflux for 6 h. The mixture was concentrated until some turbidity was observed. An amorphous white solid appeared upon cooling, which was filtered and washed with cold ethanol (*ca.* 0.5 mL). This solid was dissolved in 5% NH_3 (3 mL) and was carefully extracted with ethyl acetate (4 × 3 mL). The aqueous phase was slowly acidified with 2 M HCl until pH 1 and

evaporated to dryness. The residue was redissolved in acetone (*ca.* 4 mL), filtered and the solvent was evaporated. An amorphous white solid, mainly *S*-3-carboxyadipic acid (21 mg, 0.110 mmol, 33.90%) was obtained. This compound was identified as mainly the 3*S*-isomer by direct comparison of its optical activity ($[\alpha]_D = -4.3°$) to that reported for authentic samples [36]. It should be noted that no residual (−)-brucine could be detected either by CE or NMR.

All adsorbed/desorbed samples were prepared as already described with minor modifications [7]. Two calcites (both golden calcite from Carthage Mine (Elmwood, Tennessee) with 22 and 13 cm maximum dimension each one) and one alkali feldspar (variety "amazonite" from Ethiopia, with 8 cm maximum dimension) crystals were used for our experiments. Previously, each crystal was cleaned by ultrasonication in water, 1:1 methanol/chloroform, and water for 10 min. Each crystal face was then etched with 0.1 M HCl for 20 s and then rinsed with water.

Each crystal was then separately immersed in 0.01 M solutions of racemic 3-carboxy adipic acid, adding NaOH until pH 5.3 for feldspar or until pH 7.7 for calcite, and left undisturbed for 24 h at room temperature. The choice of pH was achieved in order to obtain a high adsorption. We postulated that the tricarboxylic acid would adsorb most readily to a positively charged mineral surface. Therefore, the pH of the solution was adjusted to be below the point of zero charge (pzc) of the mineral. In the case of calcite, with pzc >8, but which begins to dissolve readily at pH <7.5, we set the solution pH at 7.7 [37]. In the case of feldspar the pzc is not yet well determined; we assumed pzc >5.5 [38] and we set the solution pH at 5.3. After that, each crystal was removed from their 3-carboxy adipic acid solutions and rinsed with water for 10 s. Adsorbed compound from every individual face was removed by holding the crystal face horizontally and pipetting 0.02 M HCl (*ca.* 1 mL) on it for 20 s, and collecting the acid solution with the same pipette. Solutions were transferred to clean vials and evaporated with an argon stream. This protocol was repeated three times on three successive days for each crystal face.

Prior to analyze the samples by CE, the stock standard solutions of racemic 3-carboxy adipic acid and of enantiomerically enriched (−)-*S*-3-carboxy adipic acid were prepared by dissolving them in water up to a final concentration of 10^{-3} M. These solutions were stored at 5°C and diluted in water to get the concentration required just prior to use. The samples of the adsorption experiments on minerals were reconstituted in 100 μL of water. All these solutions were filtered through 0.45 μm pore size disposable nylon filters from Titan (Eatontown, NJ, USA) and analyzed immediately.

2.2 Apparatus

GC analyses were conducted in a Turbomass Gold GC-MS instrument (Perkin-Elmer, Wellesley, MA), using helium as the carrier gas. GC capillary columns with chiral stationary phases were based on β-CD (CyclosilB, Agilent J&W Scientific, Palo Alto, CA, and CYDEX B, SGE, Melbourne, Australia), γ-CD (Rt-γDEXsa, Restek, Bellefonte, PA), and chiral diamides (Permabond-L-Chirasil-Val, Macherey-Nagel, Düren, Germany).

An HP^{3D} CE system (Agilent Technologies, Palo Alto, CA, USA) equipped with an on-column DAD was employed. Instrument control and data acquisitions were performed with the HP^{3D}-CE ChemStation software. Separations were performed on different kind of capillaries: uncoated fused-silica capillaries of 50 and 75 μm id (375 μm od) with a total length of 58.5 and 80.5 cm (50 and 72 cm to the detector) respectively, were purchased from Composite Metal Services (Worcester, England), and bubble capillary of 75 μm id (375 μm od) with a total length of 80.5 cm (72 cm to the detector) from Agilent Technologies. The selected instrumental conditions were: capillary temperature, 15°C, electrokinetic injection, 20 kV for 10 s, applied voltage, −20 kV, and UV detection at 192 ± 2 nm.

2.3 Procedure

Before use, new capillaries were flushed with 1 M NaOH for 30 min and then with water for 5 min. Different conditioning protocols were carried out depending on the coating procedure. When a dynamic adsorbed coating with HDB was used, the capillaries were rinsed on a daily basis with buffer containing 0.001% HDB in the absence of VC for 30 min. Between injections, the capillary was rinsed with the same solution for 4 min before a plug of VC solution was charged into the capillary. Since the adsorption of HDB and VC onto the capillary wall is a competition process, a gradual accumulation of VC is possible, which could drop the separation efficiency and increase the time of analysis. The VC adsorbed onto the capillary wall can be cleaned by rinsing the capillary wall at the end of the day with 0.1 M H_3PO_4 followed by water for 5 min [21]. On the other hand, when a physically adsorbed coating with DMA-EpyM (40–60 w/w) was employed, each new capillary was rinsed with 0.1 M NaOH for 20 min, followed by 10 min with polymer solution (0.1 mg/mL), and keeping this solution overnight into the capillary. Before the first injection and between subsequent injections, the capillaries were rinsed with water for 2 min followed by 2 min with 0.1 mg/mL polymer solution, and next replacing this solution by flushing the capillary with buffer in abscence of VC for 2 min. Finally, a plug of VC solution was charged.

Buffer solution (0.1 M phosphate) was prepared diluting the appropriate amount of sodium dihydrogen phosphate dihydrate with Milli-Q water, and adjusting the pH to the desired value (pH 6.0) with 0.1 M sodium hydroxide. When inversion of the EOF became necessary, amounts of 0.001% HDB, itself prepared each time from a stock solution of 1% HDB, was added to the buffer. A 744 pH-meter from Metrohm (Herisau, Switzerland) was employed to adjust the pH of the separation buffers. Finally, BGEs were prepared

dissolving the appropriate amount of VC in the buffer solution. All these solutions were filtered prior to use through 0.45 μm pore size disposable nylon filters from Titan.

2.4 Data treatment

The values of resolution between adjacent peaks for the enantiomers were obtained from the corresponding migration times and their peak width at half height using the ChemStation software. All e.e. were calculated by

$$\text{e.e. } (\%) = \frac{(S-R)}{(S+R)} \times 100 \quad (1)$$

where S and R stand for the peak height of each enantiomer. Therefore, e.e.>0 shows an excess of S-enantiomer, e.e.<0 shows an excess of R-enantiomer, and e.e. = 0 shows a racemic sample.

The Poiseuille equation was employed to calculate the length of plug of VC charged into the capillary by the partial filling method:

$$l = \frac{t \cdot d^2 \cdot P}{3200 \cdot L \cdot \eta} \text{ (mm)} \quad (2)$$

where P is the injection pressure (mbar), t the time of duration of the pressure (s), d the inner diameter of the capillary (μm), L the capillary length (cm), and η the viscosity of VC dissolution (cp; the viscosity of water was selected due to small VC concentration).

Experimental data were calculated using Excel Microsoft XP®. Statistical parameters were calculated with the 5.1 Statgraphics Plus® software. Graphs with different electropherograms were composed with the 6.0 Origin® software.

3 Results and discussion

We first tried GC to resolve the 3-carboxy adipic acid racemate. Four different GC capillary columns with chiral stationary phases were tried. Two of them were based on β-CD, another was based on γ-CD, and another on chiral diamides. GC analyses were conducted in a GC-MS instrument. In our hands, no enantiomeric separation was achieved in any case. Therefore, we decided to resort to CE in order to achieved the before mentioned enantioseparation.

3.1 Development of an analytical methodology for the enantiomeric separation of 3-carboxy adipic acid by CE

The first step in the development of a chiral analytical methodology by CE is the selection of an adequate chiral selector to be added to the separation buffer. Although, to the best of our knowledge, no methods for the racemic resolution of tricarboxylic acids have been yet reported, the published chiral separations by CE of aliphatic mono- and dicarboxylic acids (see Section 1) helped us to design two main strategies to attempt the enantiomeric separation of 3-carboxy adipic acid: (i) the use of neutral CDs or (ii) macrocyclic antibiotics as chiral selectors. In addition, short-chain organic acids are small water-soluble molecules that get negatively ionized at pH values higher than 3, and do not present other chromophore more than the carboxylic group that absorbs weakly and presents its maximum absorbance around 200 nm. Direct measurements at 200 nm or below would be seen as a good option for detecting the carboxylic acid group, but this low wavelength can be only employed when working with non-UV-absorbing aqueous electrolytes. We first considered the use of a neutral CD like HP-β-CD due to its low UV absorption and lower cost than a macrocyclic antibiotic such as VC. Coated capillaries were employed in this work so that 3-carboxy adipic acid was analyzed under coelectroosmotic conditions (same direction for electroosmotic and electrophoretic mobility), which significantly shortening the analysis time [39, 40]. Dynamic coating of the capillary inner wall was achieved by adding HDB to the BGE. Since HDB is a polycationic polymer, it tends to adsorb on the capillary wall and its positive charge produces a reversal EOF which migrates in the same direction as the negatively charged analytes. After the optimization of some experimental parameters, the best conditions to achieve the chiral separation of 3-carboxy adipic acid with HP-β-CD only enabled the partial separation of the enantiomers (R_s = 0.6).

Due to the poor results obtained with HP-β-CD as the chiral selector, we turned to VC to study the possibility of increasing the obtained chiral resolution. As indicated in the introduction, VC had some drawbacks such as the adsorption on the capillary wall due to its cationic nature in the pH range from 4 to 7, and its strong absorption in the UV. In order to prevent VC from adsorbing on the capillary walls, coated capillaries were employed in this work by adding HDB to the BGE. Moreover, due to the UV absorption of this macrocyclic antibiotic, we resorted to the partial filling method, which prevents the presence of the absorbing chiral selector in the detector path. Thus, the capillary was partially filled with a BGE containing VC in a zone between the detection window and the inlet end prior to injection. Since the addition of VC to the BGE in the inlet and outlet vials was eliminated, the consumption of this expensive chiral selector was minimized. In the separation process, the zone of VC started to migrate toward the cathode and the analyte migrated to the anode. Since the chiral separation process only occurs inside a plug of the VC solution, the plug length was optimized to be long enough to give chiral separation for analytes without the interference due to the VC. To achieve this, the time needed for the partial filling of the capillary had to be optimized, which was done in two steps. First, the time required by a 10 mM VC solution (in 0.1 M phosphate buffer with 0.001% HDB) to reach the detector was measured when using a 50 μm id and 50 cm effective length (58.8 cm total length) capillary and a pressure of 50 mbar. Second, a 3-carboxy adipic acid solution was hydrodynamically injected (50 mbar × 10 s) in the capillary after introducing the plug of VC using different times,

always below the time obtained in the first step of this optimization process. A voltage of −15 kV and a temperature of 15°C were also employed. As a result, a time of 400 s was established as maximum value to avoid the interference of the VC band with the detection of the 3-carboxy adipic acid peaks. From the Poiseuille equation (Eq. 2) given in Section 2, the length of the VC band was calculated to be 26.5 cm under the described conditions.

The study of the influence of the pH on the separation of the enantiomers of 3-carboxy adipic acid was achieved in the range from 5.0 to 6.5 where VC is stable and positively charged. In addition, at these pH values, 3-carboxy adipic acid is negatively charged. Figure 2 shows that only at pH 6.0 and 6.5 good shapes for the peaks were obtained, the highest enantiomeric resolution ($R_s = 1.3$) resulting at pH 6.0. This resolution could not be improved by adding low percentages (5, 10%) of organic modifiers such as methanol or ACN. As a consequence, the effect of the VC concentration was investigated. Figure 3 shows that the enantiomeric resolution increased when increasing the VC concentration due to the higher interaction between the VC and 3-carboxy adipic acid which in turn increased the analysis time. However, Fig. 3 also shows that at a 20 mM concentration of VC, an impurity described for VC in the literature overlapped with the enantiomers [23]. Then, a 15 mM concentration of VC was chosen which enabled an enantiomeric resolution of 2.0 in an analysis time close to 8 min.

In order to know the migration order of the enantiomers of 3-carboxy adipic acid, a solution enriched in the 3*S* enantiomer (levogire) was injected. As no pure enantiomers of 3-carboxy adipic acid were commercially available, the 3*S* enantiomer had to be prepared by fractional crystallization with (−)-brucine (see Section 2). Figure 4 shows that the first

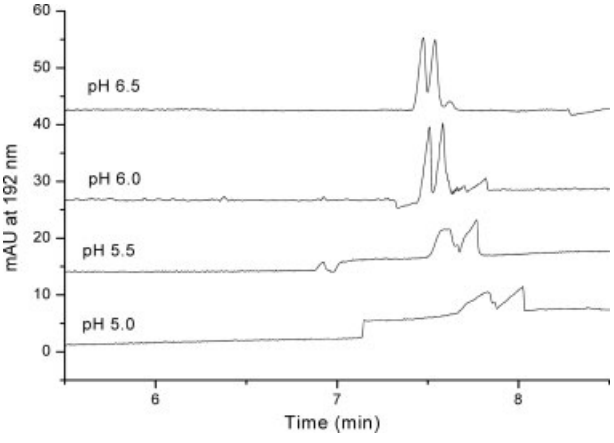

Figure 2. Variation of the enantiomeric resolution for 3-carboxy adipic acid as a function of pH using VC as chiral selector in the partial filling method. Experimental conditions: BGE, 0.1 M phosphate buffer with 0.001% HDB; partial filling, 400 s × 50 mbar of 10 mM VC in BGE. Instrumental conditions: fused-silica capillary, 58.5 cm (50 cm to the detector window) × 50 μm id; injection by pressure at 50 mbar × 10 s; applied voltage, −15 kV; temperature, 15°C; and detection at 192 ± 2 nm.

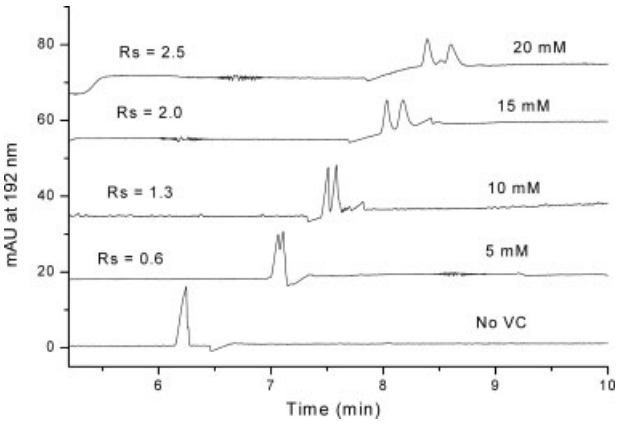

Figure 3. Variation of the enantiomeric resolution for 3-carboxy adipic acid as a function of VC concentration. Experimental conditions: BGE, 0.1 M phosphate buffer (pH 6.0) with 0.001% HDB; partial filling, 400 s × 50 mbar of different concentrations of VC in BGE. Other conditions as in Fig. 2.

and second eluting peaks respectively corresponded to the 3*S*-and the 3*R*-enantiomer. Figure 4 also shows that an increase in the injected volume (50 mbar × 15 s) was possible without a loss in the baseline resolution of the enantiomers.

3.2 Enhancement of the detection sensitivity for the enantiomers of 3-carboxy adipic acid

An estimation of the detection limit (S/N = 3) obtained under the optimized conditions gave rise to a value close to 1.25×10^{-4} M for each enantiomer. As the amount of compound in samples gave rise to a concentration smaller than this value, it was decided to enhance the detection sensitivity for the enantiomers of 3-carboxy adipic acid. Two strategies were investigated, a preconcentration by field-amplified

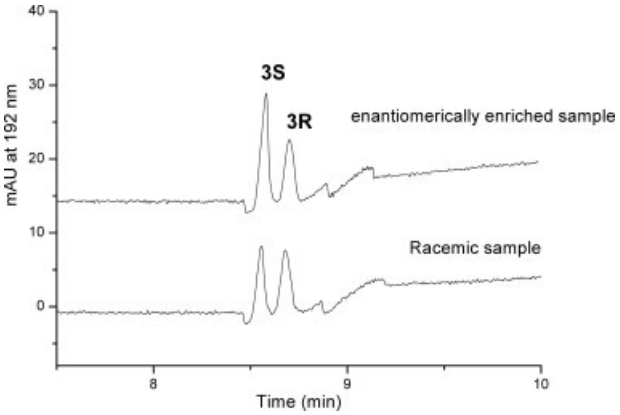

Figure 4. 3-Carboxy adipic acid analysis in an enantiomerically enriched sample and in a racemic sample. Experimental conditions: BGE, 0.1 M phosphate buffer (pH 6.0) with 0.001% HDB; partial filling, 400 s × 50 mbar of 15 mM VC in BGE. Instrumental conditions: injection by pressure at 50 mbar × 15 s. Other conditions as in Fig. 2.

sample stacking (FASS) using electrokinetic injection of the sample dissolved in water, and a new capillary with a extended light path (bubble capillary of 75 μm id × 72 cm effective length). With this capillary, we thought that an increase in the plug of VC in the partial filling method could enhance the enantiomeric resolution (selectivity), but in this case the absorption band of VC ran quite close to the peaks of the enantiomers. As a consequence, the employed filling time was also 400 s. A comparison between the electropherograms of the Figs. 5a and b showed a 50-fold increase in the sensitivity (as the ratio of the corrected areas) when employing an electrokinetic injection of −10 kV for 10 s. However, under these conditions, the loss in selectivity originated the comigration of the peaks of the enantiomers with the above-mentioned VC impurity. In order to overcome this problem, a polymer enabling the physical coating of the capillary was used instead of the dynamic modification with HDB. In fact, taking into account the need of working with very low EOF, the polymer EPyM-DMA (see Section 2) was chosen since it showed its usefulness in recent works to give low anodic EOF at pH<6 [35, 41]. Resorting to this polymer enabled the adequate discrimination between the peaks of the enantiomers and the VC impurity as shown in Fig. 5c. In addition, the observed increase in the enantiomeric resolution enabled us to increment the separation voltage up to −20 kV which decreased the analysis time. Under these conditions, the use of the bubble capillary (see Fig. 5d) enabled an 80-fold increase in the detection sensitivity with respect to that obtained in Fig. 5a. Consequently, a detection limit (S/N = 3) of 1.5×10^{-6} M for each enantiomer was thus obtained. The loss of resolution observed in Fig. 5d can be due to the higher sensitivity of the bubble capillary, which makes that the peaks are higher and there is a greater overlapping. However, since the samples have a concentration substantially less than that corresponding to Fig. 5 (more than 10 times less), the loss of resolution will be corrected as will be shown below.

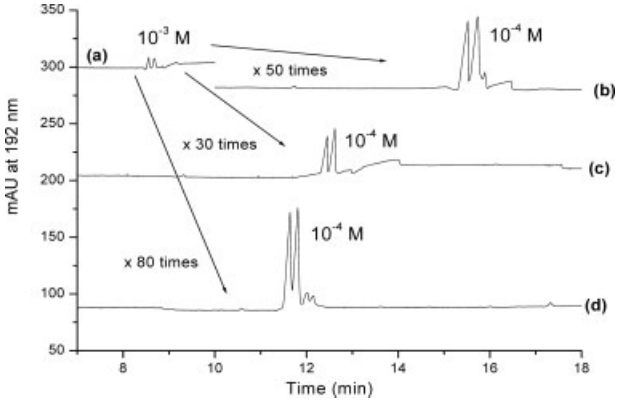

Figure 5. Strategies to increase the sensibility in the enantiomeric separation of 3-carboxy adipic acid. Same conditions in the signals: partial filling, 400 s × 50 mbar of 15 mM VC in BGE; temperature, 15°C; detection at 192 ± 2 nm. Other conditions: (a) BGE, 0.1 M phosphate buffer (pH 6.0) with 0.001% HDB; fused-silica capillary, 58.5 cm (50 cm to the detector window) × 50 μm id; applied voltage, −15 kV; injection by pressure at 50 mbar × 15 s; (b) BGE, as (a); fused-silica capillary, 80.5 cm (72 cm to the detector window) × 75 μm id; applied voltage, −15 kV; electrokinetic injection, −10 kV × 10 s; (c) BGE, 0.1 M phosphate buffer (pH 6.0), coated capillary with EpyM-DMA, 80.5 cm (72 cm to the detector window) × 75 μm id; applied voltage, −20 kV; electrokinetic injection, −10 kV × 10 s; (d) BGE, as (c); coated bubble-capillary with EpyM-DMA, 80.5 cm (72 cm to the detector window) × 75 μm id; applied voltage, −20 kV; electrokinetic injection, −10 kV × 10 s.

3.3 Determination of the enantiomeric excess for 3-carboxy adipic acid in adsorption samples on minerals

We next used our method to determine the e.e. of samples of 3-carboxy adipic acid prepared in adsorption experiments on calcite and alkaline feldspar (see Section 2). However, when one of these samples was analyzed (see electropherogram in Fig. 6a), the peaks of the enantiomers were very small and difficult to detect. In order to achieve the sensitivity needed to measure the enantiomeric excess of the acid in the adsorption samples on minerals, a 12-fold increase in the electrokinetic injection (from −100 to −1200 kV) was performed. This injection originated a ∼1000-fold increase in sensitivity (LOD ∼ 10^{-7} M) in relation to the conditions previously optimized (injection by pressure at 50 mbar × 15 s) and enabled the determination of the enantiomeric excess of 3-carboxy adipic acid in the studied samples (at subnanomol level). Electropherograms obtained for these samples are shown in Figs. 6b (alkali feldspar) and 6c (calcite) as examples. Final experimental conditions employed to analyze these samples are summarized in Table 1 which shows the

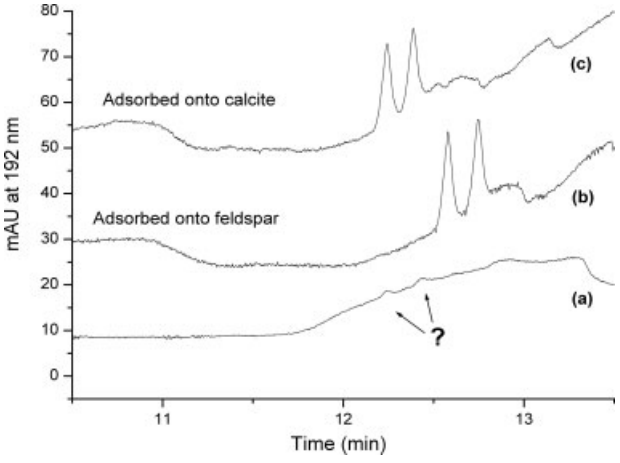

Figure 6. Adsorption of 3-carboxy adipic acid onto minerals. Experimental conditions: BGE, 0.1 M phosphate buffer (pH 6.0); partial filling, 400 s × 50 mbar of 15 mM VC in BGE. Instrumental conditions: coated bubble-capillary with DMA-EpyM, 80.5 cm (72 cm to the detector window) × 75 μm id; temperature, 15°C; applied voltage, −20 kV; detection at 192 ± 2 nm; electrokinetic injection, (a) −10 kV × 10 s, (b) and (c) −20 kV × 60 s.

Table 1. Enantiomeric excess (e.e.) of adsorbed samples (mean value and SD, $n = 3$), and *t*-test to compare the mean of enantiomeric excess of a racemic sample ($n = 9$) with the enantiomeric excess of the different adsorption samples ($n = 3$)

Samples	Face designation	Faces' Miller indices[a)]	e.e. ± s (%)	$t_{calculated}$ $t_{tabulated} = 2.228$ ($n = 10$; $\alpha = 0.05$)	Type chiral faces[b)]
Feldspar	F1	(110)	10.5 ± 0.4	10.461	*S*
	F2	(1-10)	−9.5 ± 0.4	9.197	*R*
	F3	(-210)	−1.7 ± 0.6	1.593	0
Calcite I	C1	(3-14)	7.1 ± 1.3	5.111	*S*
	C2	(214)	−2.7 ± 0.7	2.501	*R*
	C3	(-324)	−2.7 ± 0.7	2.501	*R*
	C4	(1-24)	7.9 ± 1.1	5.823	*S*
	C5	(1-34)	−4.2 ± 0.8	3.644	*R*
	C6	(1-14)	2.5 ± 0.5	2.495	*S*
	C7	(-114)	1.0 ± 0.8	0.996	0
	C8	(014)	−0.3 ± 1.1	0.112	0
Calcite II	C1	(3-14)	5.8 ± 2.8	4.490	*S*
	C2	(214)	0.6 ± 1.3	0.655	0
	C3	(-234)	7.7 ± 1.4	7.124	*S*
	C4	(1-24)	6.6 ± 0.7	6.469	*S*
	C5	(1-14)	1.0 ± 1.8	1.003	0
	C6	(-114)	2.2 ± 0.9	2.209	0

Experimental conditions: BGE, 0.1 M phosphate buffer (pH 6.0); partial filling, 400 s × 50 mbar of 15 mM VC in BGE. Instrumental conditions: coated bubble-capillary with DMA-EpyM, 80.5 cm (72 cm to the detector window) × 75 μm id; electrokinetic injection, −20 kV × 60 s; temperature, 15°C; applied voltage, −20 kV; detection at 192 ± 2 nm.

a) The designation of face (214) is arbitrary. However, once one face is designated as the (214), all other Miller indices are defined by their relative orientation to face (214) [42].

b) Type "0" are achiral faces, which display no chiral selectivity.

results obtained for the enantiomeric excess of 3-carboxy adipic acid in the adsorption samples on calcite and alkali feldspar. These values were calculated from peak heights and not peak areas since a better accuracy (close to the expected value for a racemic sample, ratio between peaks = 1) and precision were obtained. In fact, the results corresponding to the measurement of the ratio between peaks of a racemic sample were 1.02 ± 0.06 using peak heights (average ± SD) and 1.1 ± 0.1 using peak areas (average ± SD).

A statistical study of the results by means of an "*F*-test" showed that there were not statistical differences between the variances obtained for the analysis of the racemic mixture (SD, 1.7%) and each of the adsorption samples (SDs, between 0.4 and 2.8%). The values obtained for the statistic parameter obtained with a "*t*-test" to compare the mean of the enantiomeric excess for a racemic sample (confidence interval for the e.e. = −0.11 ± 1.31% at the 95.0% confidence level) with the enantiomeric excess for the different adsorption samples are also included in Table 1. Comparison between calculated and tabulated "*t*" values indicated that there was a statistically significant enantioselective adsorption of 3-carboxy adipic acid on some mineral samples.

As shown in Table 1, in the case of feldspar, the (110) face according to its Miller indices [43] (here designated F1) is chiral and displays selective adsorption of the *S* enantiomer ("*S*" face). The (1-10) face (designated F2) is oppositely chiral, with the R enantiomer being selectively adsorbed ("*R*" face). The (-201) (designated F3) is achiral and displays no chiral selectivity. With respect to calcite samples, for calcite I, the selective adsorption of S and R enantiomers also occurred depending on the crystal faces. The (3-14), (-1-24) and (1-14) faces (designated C1, C4 and C6, respectively) are "*S*" faces, while the (214), (-324) and (1-34) faces (designated C2, C3, and C5, respectively) are "*R*" faces. On the other hand, the (-114) and (014) faces (designated C7, and C8, respectively) are achiral and display no selective adsorption. In the case of calcite II, the (3-14), (-234), and (-1-24) faces (designated C1, C3, and C4, respectively) are "*S*" faces, while the (214), (1-14), and (-114) faces (designated C2, C5, and C6, respectively) are achiral faces. The observed resultsw are in agreement with those described in the literature [7,8,], with two exceptions. First, the C6 face in calcite I that gave a small enantioselective adsorption of S enantiomer (e.e. = 2.5%) and it must be achiral as the C5 face in calcite II since it is a (1-14) face. Second, the C2 face in calcite II that being a (214) face must have an enantioselective adsorption of R enantiomer as the C2 face in calcite I.

4 Concluding remarks

The separation of the enantiomers of a short-chain tricarboxylic acid (3-carboxy adipic acid) has been performed for the first time in this work using CE with UV detection without sample derivatization in spite of its low absorption of the UV light. The use of VC as chiral selector in the separation buffer using the partial filling technique has enabled the separation of the two enantiomers of the acid in about 8 min with a resolution of 2.0. The fact that the chiral separation of this acid was not possible by other analytical separation techniques such as GC shows the high potential of CE for chiral separations. In our hands, the racemic resolution of 3-carboxy adipic acid could not be accomplished by GC means using several chiral stationary phases (data not shown).

In addition, the use of a low detection wavelength (192 nm) together with a preconcentration by FASS and a bubble capillary enabled us to determine the enantiomeric excess adsorption of 3-carboxy adipic acid on calcite and alkali feldspar crystals at 10^{-6} M concentration levels. Results showed that an enantioselective adsorption of the enantiomers of 3-carboxy adipic acid on minerals took place. These results have implications for rationalizing origin of life scenarios, since calcite and feldspar may have provided a plausible mechanism for asymmetrically selecting and concentrating the D- and L-enantiomers of amino acids and other organic compounds. This may point to a possible mechanism for prebiotic origins of biological homochirality.

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