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Short Communication

High-performance liquid chromatographic separation of fullerenes (C_{60} and C_{70}) using chemically bonded γ-cyclodextrin as stationary phase

K. Cabrera*, G. Wieland and M. Schäfer

E. Merck, R&D Chromatography, Frankfurter Strasse 250, 6100 Darmstadt (Germany)

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ABSTRACT

γ-Cyclodextrin chemically bonded to silica was used as a stationary phase for the HPLC separation of the two fullerenes, C_{60} and C_{70}. C_{70} is much more strongly retarded than C_{60} on this stationary phase. Chromatography on the corresponding unmodified silica showed no separation of the two fullerenes, indicating that the separation is due to the selective interaction with the γ-cyclodextrin moieties.

INTRODUCTION

The discovery of buckminsterfullerene (C_{60}) and the more recent development of methods to obtain fullerenes in larger amounts have opened up a highly active field of research [1,2]. Numerous scientists are now working on the synthesis and structural characterization of new fullerenes [3–5]. For all these investigations, chromatographic methods for the purification and analysis of fullerene mixtures are needed. So far, fullerenes have been successfully chromatographed on typical normal-phase systems using silica [3,6], alumina [7] or graphite [8] as the stationary phase and *n*-hexane as the mobile phase. Reversed-phase silica with C_{18} modification [9,10] and the Pirkle type of stationary phase (N-3,5-dinitrobenzoylphenylglycine bonded to aminopropylsilica) [6,11] have also been used for the chromatographic separation of fullerenes. The latter shows high selectivity for the separation of the C_{60} and C_{70} compounds. Therefore, semi-preparative separations have also been performed on this type of phase. Recently, a synthetic polymer (polystyrene) has been used as a stationary phase for the gel permeation chromatography of fullerenes [12].

Cyclodextrins are cyclic oligosaccharides which are known for their ability to form inclusion complexes with different substrates [13]. As a result of this property, cyclodextrins can differentiate between structural, geometrical and optical isomers. This has been used for the chromatographic separation of compounds either by adding cyclodextrins to the mobile phase [14,15] or by their chemical bonding to the stationary phase [16,17]. Recently, γ-cyclodextrin has been used as a complexing reagent for C_{60}, resulting in a water-soluble fullerene [18].

* Corresponding author.

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The incorporation of C_{60} in azacrown ethers which are oriented in a monolayer has also been reported [19]. It was therefore of interest to see whether γ-cyclodextrin chemically bonded to silica (ChiraDex-GAMMA) could be a specific stationary phase for the chromatographic separation of fullerenes.

In this paper, the efficient HPLC separation of C_{60} and C_{70} fullerenes on this stationary phase is described.

EXPERIMENTAL

Chemicals and reagents

A C_{60}–C_{70} fullerene mixture was obtained from Aldrich (Steinheim, Germany). Pure C_{60} was supplied by Fluka (Neu-Ulm, Germany). *n*-Hexane and toluene used for chromatography were of LiChrosolv grade (Merck, Darmstadt, Germany).

HPLC columns

LiChroCART LiChrospher Si 100, 5 μm (250 mm × 4 mm I.D.), and LiChroCART ChiraDex-GAMMA (γ-cyclodextrin chemically bonded to silica), 5 μm (250 mm × 4 mm I.D.), were obtained from Merck.

HPLC instrument

All chromatographic separations were carried out on a Merck–Hitachi HPLC system with a Rheodyne injection valve, an L-6200 intelligent pump, an L-4000 detector and a D-2500 integrator. Temperature experiments were performed with an Inlabo F 10-UC thermostat.

RESULTS AND DISCUSSION

γ-Cyclodextrin chemically bonded to silica (ChiraDex-GAMMA) was investigated as a stationary phase for the chromatographic separation of C_{60} and C_{70} with *n*-hexane as the mobile phase (Fig. 1a). A single injection of C_{60} onto ChiraDex-GAMMA showed that C_{70} is eluted after the C_{60} fullerene with a difference of almost 40 min in retention time. This indicates a much stronger interaction of C_{70} with the stationary phase. Attempts to separate the fullerene mixture on the corresponding unmodified

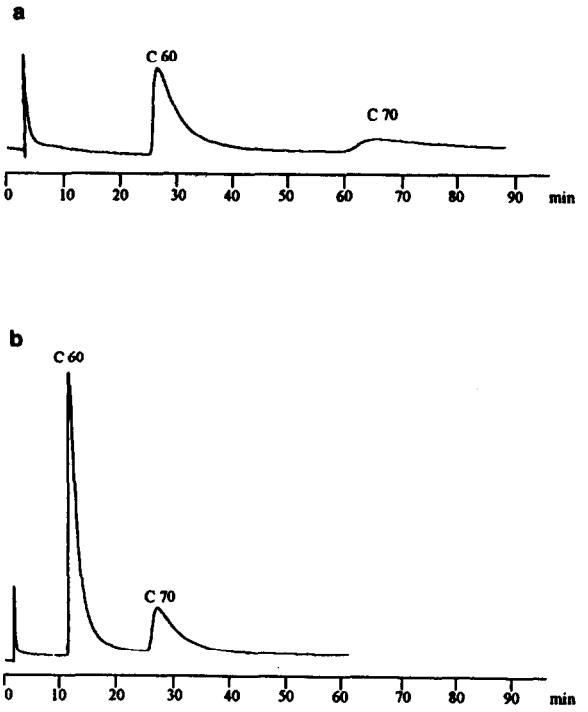

Fig. 1. Separation of C_{60} and C_{70} on ChiraDex-GAMMA. Chromatographic conditions: (a) mobile phase, *n*-hexane; flow-rate, 1 ml/min; temperature, room temperature; detection, UV at 298 nm; injection volume, 10 μl; sample, 2 mg of C_{60}–C_{70} (*ca.* 10:1) dissolved in 10 ml of toluene; (b) as (a) except flow-rate, 2 ml/min and temperature, 30°C.

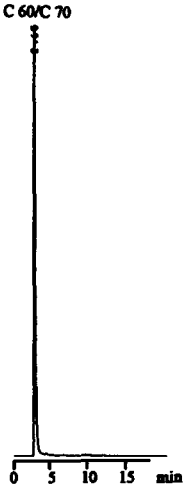

Fig. 2. Chromatography of C_{60}–C_{70} on LiChrospher Si 100. Chromatographic conditions: mobile phase, *n*-hexane; flow-rate, 1 ml/min; temperature, room temperature; detection, 334 nm; injection volume, 10 μl; sample, 2 mg of C_{60}–C_{70} (*ca.* 10:1) dissolved in toluene.

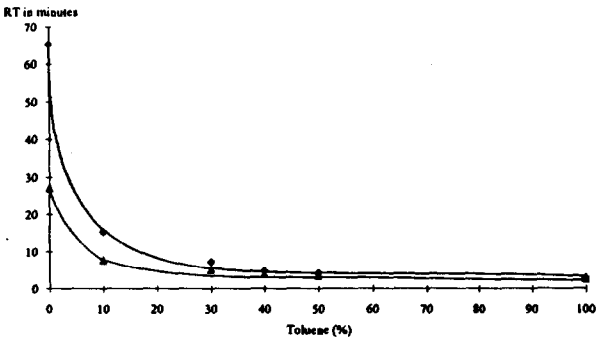

Fig. 3. Influence of the amount of toluene in the mobile phase on the separation of (▲) C_{60} and (◆) C_{70} on ChiraDex-GAMMA. Chromatographic conditions: mobile phase, *n*-hexane–toluene; flow-rate, 1 ml/min; temperature, room temperature; detection, 334 nm; injection volume, 10 μl; sample, 2 mg of C_{60}–C_{70} (*ca.* 10:1) dissolved in toluene. RT = Retention time.

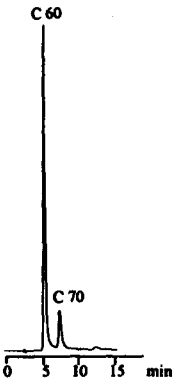

Fig. 4. Separation of C_{60}–C_{70} on ChiraDex-GAMMA. Chromatographic conditions: mobile phase, *n*-hexane–toluene (70:30, v/v); flow-rate, 1 ml/min; temperature, room temperature; detection, 334 nm; injection volume, 10 μl; sample, 2 mg of C_{60}–C_{70} (*ca.* 10:1) dissolved in toluene.

silica (LiChrospher Si 100) under comparable conditions failed (Fig. 2). Therefore, it is clear that the retention and separation of the two fullerenes is due to interactions with the γ-cyclodextrin moieties of the stationary phase. However, the previously described separation is not optimum for routine analyses, because of the long retention times and strong peak broadening. As expected, an increase of temperature (30°C) and flow-rate (2 ml/min) resulted in a considerable decrease in the retention times (Fig. 1b).

Despite the good separation of the two fullerenes under these conditions, the poor solubility of the compounds in *n*-hexane makes this separation useful only on an analytical scale. As toluene is one of the best solvents for fullerenes, the influence of toluene as a component of the mobile phase on the separation of ChiraDex-GAMMA was studied. An increase in toluene concentration in the mobile phase caused a strong decrease in the retention times and eventually resulted in a loss of separation (Fig. 3). These results indicate that there is competition between toluene and fullerene molecules for the interaction with the stationary phase. However, a mobile phase containing 30% (v/v) of toluene in *n*-hexane gave a sufficient difference in the retention times to allow a good separation of C_{60} and C_{70} (Fig. 4). Under these conditions it would be possible to perform separations on a preparative scale.

Concerning the mechanism leading to the separation of C_{60} and C_{70} on ChiraDex-GAMMA, at present we can only suggest the following interpretation. Chromatographic experiments have clearly shown that the γ-

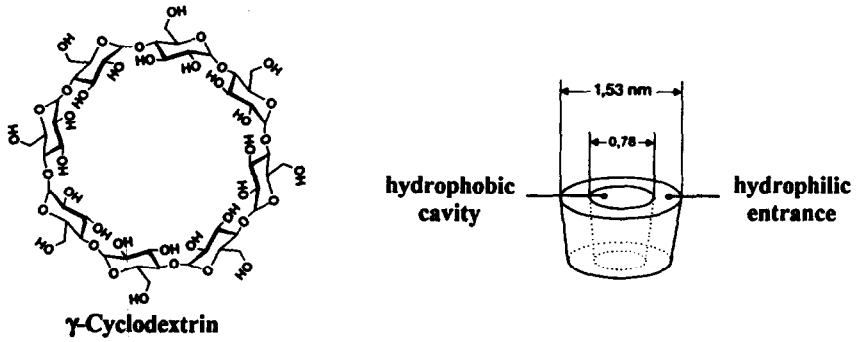

Fig. 5. Chemical and geometrical structures of γ-cyclodextrin.

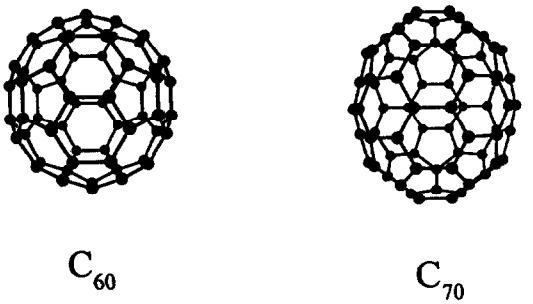

Fig. 6. Chemical and geometrical structures of C_{60} (7.1 Å diameter [20]) and C_{70} (6.9 Å diameter at centre, 7.8 Å longitudinal axis [21]).

cyclodextrin moieties of the stationary phase are responsible for the separation of C_{60} and C_{70} fullerenes. As γ-cyclodextrin is a cyclic oligosaccharide, formed by eight chiral D-glucose molecules (Fig. 5), it possesses a cavity, in which molecules of different structures can be included [13]. Therefore, it is possible that the fullerene molecules form at least partial inclusion complexes with γ-cyclodextrin. This would explain the retardation of C_{60} and C_{70} on ChiraDex-GAMMA in contrast to almost no retention and consequently no separation on the corresponding unmodified silica stationary phase. Moreover, the observed decrease in retention times caused by toluene, which was added to the mobile phase, can be explained by competitive interactions of toluene with the γ-cyclodextrin molecule, thereby occupying the cavity. These explanations, however, give no answer as to why C_{70} is much more strongly retarded than C_{60} in the presence of γ-cyclodextrin moieties. C_{60} and C_{70} are, however, different in size and shape (Fig. 6). It may therefore be that the geometry of C_{70} is more favourable for the interaction with the γ-cyclodextrin molecules.

In conclusion, γ-cyclodextrin chemically bonded to silica (ChiraDex-GAMMA) is another highly selective stationary phase for the HPLC separation of C_{60} and C_{70} fullerenes. As the separation of these two fullerenes is probably due to the formation of at least partial inclusion complexes with γ-cyclodextrin, which is a chiral moiety, it would be very interesting to try the separation of other fullerenes, especially chiral molecules [4].

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