Non-aqueous capillary electrophoresis separation of fullerenes and C60 fullerene derivatives.

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Abstract

As the interest in the use of fullerene compounds in biomedical and cosmetic applications increases, so too does the need to develop methods for their determination and quantitation in such complex matrices. In this work we studied the behavior of C60 and C70 fullerenes in non-aqueous capillary electrophoresis, as well as two C60 fullerene derivatives not previously reported by any electrophoretic method, N-methyl-fulleropyrrolidine (C60 pyrr) and (1,2-methanofullerene C60)-61-carboxylic acid (C60-COOH). . The separation was performed using fused-silica capillaries with an I.D. of 50 µm and tetraalkylammonium salts, namely tetra-n-decylammonium bromide (TDAB, 200 mM) and tetraethylammonium bromide (TEAB, 40 mM), in a solvent mixture containing 6% methanol and 10% acetic acid in acetonitrile:chlorobenzene (1:1 *v/v*) as the background electrolyte (BGE). Detection limits, based on a signal-to-noise ratio of 3:1, were calculated and values between 1 and 3.7 mg/L were obtained. Good run-to-run and day-to-day precisions on concentration were achieved with RDSs lower than 15%. For the first time, an electrophoretic technique (NACE) has been applied for the analysis of C60 fullerene in a commercial cosmetic cream. A standard addition method was used for quantitation and the result was compared with that obtained by analyzing the same cream by LC-MS.

Keywords Fullerenes, Tetraalkylammonium salts, Non-aqueous capillary electrophoresis, Commercial cosmetic cream

Introduction

Fullerenes are spherical carbon nanoparticles with a unique cage structure. Their presence in the environment is due to both natural and anthropogenic phenomena such as volcanic eruptions, forest fires and the combustion of carbon-based materials. Since their discovery in 1985, fullerenes have attracted considerable attention in different fields of science. The fullerene family, and especially C60 fullerene, has very appealing photo-, electro-chemical and physical properties, which can be exploited in many different fields. Their electron double bonds allow pristine fullerenes, which are intrinsically hydrophobic [1], to become readily derivatized and water soluble through the addition of various functional groups. Hence, these molecules are increasingly being investigated for use in biomedical, cosmetic and industrial applications, ranging from drug-delivery systems and anti-aging formulations to electrical components [2]. The size, hydrophobicity, three-dimensionality and electronic configuration of fullerenes make them an appealing subject in medicinal chemistry. Their unique structure, coupled with their immense scope for derivatization, make them potential therapeutic agents. C60 fullerene is also used in a variety of personal care products, although its widespread usage is a recent development [3]. Among the numerous derivatives of C60 fullerene, fulleropyrrolidines play an important role in the controllable synthesis of new materials and biologically active compounds [4].

Although significant advances have been made in the analysis of fullerenes in the past few years [5], there is still a need to develop effective, sensitive and reliable analytical methods for their determination. Reversed-phase liquid chromatography coupled to mass spectrometry (LC-MS) has been the method of choice for analyzing them in different matrices such as environmental waters [6,7], biological fluids [8,9] and cosmetics [10,11]. These studies have focused primarily on the analysis of C60 and C70 fullerenes, although some have also analyzed fullerene derivatives such as N-methyl-fulleropyrrolidine [6] and [6,6]-phenyl C61-butyric acid methyl ester [7].

Capillary electrophoresis (CE) techniques have also been used to analyze fullerenes, especially fullerene derivatives [12-18]. For instance, the electrophoretic behavior of a highly water-soluble anionic dendro[60]fullerene derivative in capillary zone electrophoresis (CZE) has been evaluated [13] and this technique has also been proposed for the analysis of Th-symmetric fullerenehexamalonic acid ($C66(COOH)_{12}$) as an alternative to ion-chromatography [14]. Micellar electrokinetic chromatography (MEKC) and CZE have been applied to the analysis of dendro[60]fullerene and carboxyfullerene ($C60(COOH)_6$) in human serum [15], and Treubig and Brown [16] showed that MEKC can be used to analyze C60 and C70 fullerenes in an aqueous buffer using sodium dodecylsulfate as a surfactant. In addition, non-aqueous capillary electrophoresis (NACE) has been reported to separate three open-cage fullerenes using trifluoroacetic acid and sodium acetate in a mixture of acetonitrile and methanol [17], and Wan and Leung presented a communication [18] showing that NACE is also suitable for the separation of C60, C70 and C84 fullerenes and several C60 fullerene derivatives using tetraalkylammonium ions in a solvent mixture containing acetonitrile, chlorobenzene, acetic acid and methanol as the background electrolyte. Nevertheless, the performance and applicability of the method were not assessed. Moreover, none of the developed NACE methods have been applied to the determination of fullerenes in real samples.

The aim of this work is to study the behavior in non-aqueous capillary electrophoresis of C60 and C70 fullerenes, as well as two C60 fullerene derivatives of relatively high hydrophobicity, N-methyl-fulleropyrrolidine (C60 pyrr) and (1,2-methanofullerene C60)-61-carboxylic acid (C60-COOH), not previously studied by electrophoretic techniques, . The composition of the background electrolyte was optimized in order to achieve the best separation of the fullerenes studied. The effect of tetraalkylammonium salts, such as TDAB and TEAB, and organic solvents on the electrophoretic behavior of fullerenes will be discussed in depth. The instrumental quality parameters of the proposed NACE method, such as limits of detection (LODs), limits of quantitation (LOQs),

linearity, and run-to-run and day-to-day reproducibility will be assessed and the method will be applied for the first time to the analysis of C60 fullerene in a commercial cream.

Materials and methods

Chemicals and standard solutions

C60 (CAS: 99685-96-8) and C70 (CAS: 115383-22-7) fullerenes and the C60 fullerene derivatives N-methyl-fulleropyrrolidine (C60 pyrr, CAS: 151872-44-5) and (1,2-methanofullerene C60)-61-carboxylic acid (C60-COOH, CAS:155116-19-1) were purchased from Sigma Aldrich (Steinheim, Germany). The chemical structures and abbreviations of these compounds are given in Figure 1. TDAB (CAS: 14937-42-9), TEAB (CAS: 71-91-0), HPLC-grade chlorobenzene, acetonitrile and methanol were also purchased from Sigma Aldrich, and acetic acid (100%), sodium hydroxide (99%) and hydrochloric acid (25%) were obtained from Merck (Darmstadt, Germany). All reagents and chemicals were of analytical grade.

Water was purified using an Elix 3 coupled to a Milli-Q system (Millipore, Bedford, MA, USA) and filtered using a 0.22 µm nylon filter integrated into the Milli-Q system.

Stock standard solutions of C60, C70, C60 pyrr and C60-COOH (~1000 mg/L) were prepared in chlorobenzene for NACE analysis and in toluene for the LC-MS method and refrigerated at 4 ˚C. Prior to analysis, each stock solution was diluted using the BGE to form the working solution.

Instrumentation

NACE experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis instrument (Fullerton, CA, USA) equipped with a diode array. Electrophoretic separations were carried out using uncoated fused-silica capillaries (Beckman) with a total length of 60 cm (50 cm effective length) x 50 µm I.D. (375 µm O.D.) and using a 200 mM TDAB, 40 mM TEAB, 6% MeOH and 10% acetic acid in a solution of acetonitrile and chlorobenzene at 1:1 ratio (*v/v*) as the background electrolyte (BGE). The capillary temperature was held at 25 ˚C. The BGE was filtered through a 0.45 µm nylon membrane filter before use. A capillary voltage of +30 kV was applied for the separation. Sample introduction was performed by hydrodynamic injection (5 s, 13.5 kPa). Direct UV detection was performed at 350 nm. The CE instrument was controlled using Beckman 32 Karat software version 5.0.

LC-MS experiments for method validation were performed following a previously described method [19]. An ultra-high performance liquid chromatography (UHPLC) system (Accela system, Thermo Fisher Scientific, San Jose, CA, USA) equipped with a quaternary pump, autosampler and column was used. The chromatographic separation was performed in a Hypersil GOLD C18 (150 mm x 2.1 mm I.D, 1.9 µm particle size) column using a solution of toluene and methanol (45:55 *v/v*) as mobile phase, isocratic elution at a flow rate of 500 µL/min and column temperature of 25 ºC. The UHPLC system was coupled to a TSQ Quantum Ultra AM (Thermo Fisher Scientific) triple quadrupole mass spectrometer and a Finnigan Ion Max source (Thermo Fisher Scientific) equipped with a Syagen PhotoMate APPI VUV light source (a krypton discharge lamp, 10 eV) (Syagen Technology, Inc., Tustin, CA, USA), and an APCI probe, which was used as nebulization-desolvation device (no corona discharge was applied). Nitrogen (purity >99.98%) was used as a sheath gas and an auxiliary gas at a flow rate of 60 and 25 a.u. (arbitrary units). Ion sweep gas was kept at 2 a.u. The temperatures of the ion transfer tube and vaporizer were both set at 350 ºC.

Capillary conditioning

New capillaries were pre-treated with 0.1 M HCl for 30 min, water for 30 min, 1 M NaOH for 30 min, and finally washed with water for 30 min. At the beginning of each session, the capillary was rinsed with 0.5 M NaOH for 15 min, water for 15 min, ACN for 5 min and the BGE for 15 min. The capillary was rinsed with the BGE for 5 min between runs and stored after rinsing with ACN and water at the end of each session. In order to increase migration time reproducibility, the capillary was post-washed with ACN for 5 min, 0.5 M NaOH for 5 min, water for 5 min, ACN for 5 min and the BGE for 5 min after several runs.

Sample preparation

A previously described method [11], with some modifications, was used to extract fullerene C60 from a personal care product (a cream). Briefly, extraction was performed by sonicating 3 g of cosmetic sample in 20 mL toluene for at least 4 h. The toluene extract was then centrifuged at 4500 rot/min for 15 min using a Selecta Centronic Centrifuge (Barcelona, Spain). The clear toluene supernatant was then evaporated to dryness and reconstituted with 200 µL chlorobenzene and injected into the CE system.

For the LC-MS method, the same extraction procedure was used but the final extract was reconstituted in toluene and methanol (20:80 *v/v*).

Results and discussion

Optimization of NACE separation

As mentioned in the introduction, only two studies using NACE for the separation of fullerene-based compounds have been published, and only one [18] reported the separation of pristine fullerenes. This last study used tetraalkylammonium salts in an organic solvent mixture containing acetonitrile, chlorobenzene, acetic acid and methanol. In the present work, the same solution was used as the preliminary BGE (100 mM TDAB, 50 mM TEAB, 6% MeOH and 10% acetic acid in acetonitrile and chlorobenzene at a ratio of 1:1 (*v/v*)), and the effect of its composition on the separation of C60 and C70 fullerenes, together with two C60 fullerene derivatives (C60 pyrr and C60-COOH) was evaluated.

It has been reported that NACE behavior of pristine fullerenes, which are neutral in the organic solvent mixture used, depends on the solvophobic interaction between the fullerenes and the tetraalkylammonium salts, such as TDAB [18]. The charged complexes of the compounds formed by the interaction between the long alkyl chains of TDAB and the fullerenes led to their electrophoretic migration under normal polarity conditions. In this paper, the effect of the background electrolyte TDAB concentration on the separation of the fullerenes studied was evaluated from 90 to 250 mM and the electropherograms obtained for each condition are shown in Figure 2. As shown, the electrophoretic migration of fullerenes is related to their hydrophobicity. The higher the hydrophobicity of the fullerenes, the stronger the interaction with TDAB, and therefore lower migration times were observed. This explains why pristine C70 fullerene (peak 1) migrate faster than pristine C60 fullerene (peak 2), which is smaller. In contrast, C60 fullerene derivatives, which have a higher polarity, showed lower electrophoretic mobility than pristine fullerenes, with C60-COOH (the most polar compound) showing the highest migration time (peak 4). The increase in TDAB concentration also improved the electrophoretic separation of the fullerenes. A slight improvement in C60 and C70 electrophoretic peak resolution with TDAB

concentration was observed (Rs values from 0.9 at 90 mM TDAB to 1.2 at 200 mM TDAB), as was an improvement in peak signal (Table1). In contrast, the migration behavior of C60 fullerene derivatives was considerably affected by TDAB, which resulted in better separation at high concentrations, although the analysis time increased noticeably. A TDAB concentration of 200 mM was selected as optimum for the BGE as a compromise between peak height, electrophoretic separation and analysis time.

The presence of a quaternary ammonium salt with short alkyl chains (TEAB) was needed to improve the resolution of pristine fullerenes. In this study, the effect of TEAB concentration, from 0 to 60 mM, on the fullerene migration time was evaluated and the observed apparent electrophoretic mobility is summarized in Table 2. As shown, the apparent mobility of the fullerenes decreased with TEAB concentration, thus improving electrophoretic separation. This is probably due to a reduction in the EOF caused by the adsorption of this salt on the internal surface of the silicacapillary tube by electrostatic interaction reducing its surface charge or zeta potential [20,21]. As a compromise between analysis time and good electrophoretic separation, a TEAB concentration of 40 mM was selected as optimum for further studies.

The composition of the organic solvent mixture used for the analysis of fullerenes by NACE was also evaluated during this study. It was observed that the volume ratio of acetonitrile and chlorobenzene exerted a certain effect on the separation. For instance, increasing the acetonitrile content (acetonitrile:chlorobenzene 3:2, *v/v*) reduced the migration time of all compounds because of the increase in EOF, which impaired the separation. For a mixture of ACN and chlorobenzene at a ratio of 2:3 (*v/v*), the migration times of the compounds increased considerably (27 min and 35 min for C60 and C60-COOH, respectively). For this reason, a solution of acetonitrile and chlorobenzene at a ratio of 1:1 (*v/v*) was maintained for further studies.

The effect of acetic acid content, from 6 to 20 % in the organic solvent mixture, on the separation of fullerene compounds was studied. It was observed that migration times increased considerably with acetic acid as its high viscosity led to a reduction in EOF (Table 3). An

improvement on C60 and C70 resolution from 6 to 10 % was observed. However, the separation of the two C60 derivatives worsened with the acetic acid content and was completely lost when 20% was used. As a compromise between analysis time and separation, an acetic acid content of 10% was chosen as the optimum value for further studies.

In this work MeOH was used in order to reduce analysis time. The effect of MeOH content in the BGE on fullerene separation is given in Table 3. As it can be seen, migration times of C60-COOH decreased with the MeOH which can be explained by hydrogen bonding interactions, reducing its polarity and so improving the interaction with TDAB. A content of 6 % methanol in the BGE was chosen as optimum value providing good peak resolution and reducing the analysis time.

In summary, 200 mM TDAB, 40 mM TEAB, 6% methanol and 10% acetic acid in a solvent mixture of acetonitrile and chlorobenzene at a ratio of 1:1 (*v/v*) was selected as the optimum BGE solution for the NACE separation of the four fullerenes studied in this work. The electrophoretic separation achieved under the optimal conditions is shown in Figure 4a.

Method performance

Instrumental quality parameters of the proposed NACE method were determined and the figures of merit are given in Table 4. Limits of detection (LODs), based on a signal-to-noise ratio of 3:1, were calculated using standard solutions prepared in chlorobenzene at low concentration levels. The LODs of the compounds ranged from 1 to 3.7 mg/L, while LOQs, based on a signal-to-noise ration of 10:1, were between 3.0 and 11.1 mg/L.

Calibration curves based on peak areas at a working range of between 3.7 and 100 mg/L were obtained and good linearity with correlation coefficients (r^2) higher than 0.98 was achieved. Run-to-run and day-to-day precisions for the fullerenes studied were calculated at two concentration levels, a low level (LOQ) and a medium level (50 mg/L), and the results, expressed as relative

standard deviation (%RSD), are also given in Table4. To obtain the run-to-run precision, a total of five replicate determinations for each concentration level were carried out on the same day (n=5). The day-to-day precision was calculated by performing five replicate determinations of each concentration level on three non-consecutive days (five replicates each day, n=15). As can be seen, acceptable run-to-run and day-to-day precisions (with RSD values lower than 14.5 %) were achieved. Run-to-run and day-to-day precisions of the migration times for all fullerenes studied were also calculated and RSD values lower than 1.8% were obtained.

In summary, the performance achieved with the optimized NACE method is adequate in terms of repeatability and reproducibility for the analysis of the fullerenes studied.

Application

In order to evaluate the applicability of the optimized NACE method, the analysis of real samples was considered. Due to the limits of detection of the NACE method, real samples with relatively high fullerene concentrations were needed. Recently, the analysis of fullerene C60 in personal care products by LC–MS was described and fullerene concentrations between 1 and 6.8 mg/L for C60 [10,11] were found. For this reason, we evaluated the applicability of the proposed NACE method by analyzing C60 in a commercial cosmetic cream.

Sample extraction was performed following a previously described procedure [11], as indicated in the experimental section. After sample extraction, extracts were submitted to the proposed NACE method and the electropherogram obtained is shown in Figure 4b. As shown, only one peak was observed, and this was identified as C60 fullerene by comparison of UV spectra and the addition of C60 standard to a cream also submitted to the extraction procedure (Figure 4b). Sample quantitation was carried out by triplicate using a standard addition method (since no blank cream samples were available), and the concentration found was 2.10±0.20 mg/L. In order to validate the NACE method for the analysis of C60 in creams, the result was compared to that obtained by analyzing the same

cream sample by LC-MS. C60 extraction from the cream was performed using the same extraction procedure but the final extract was reconstituted in toluene and methanol at a ratio of 20:80 (*v/v*). Quantitation was also performed by triplicate and the concentration found was 1.93±0.15 mg/L. The LC-MS chromatogram obtained for the quantitation of C60 in a commercial cometic cream is shown in Figure 3(c). A statistical paired-sample comparison analysis was performed based on the quantitation results obtained in both the NACE and LC-MS methods. For a 95% confidence level, the results were not significantly different (*p*-value of 0.30). The results obtained showed that the NACE method proposed in this work is suitable for the analysis of C60 in commercial cosmetic creams. This method is less expensive and less contaminant, because of the use of much lower amount of solvents, than LC-MS methods which for this kind of application require the use of high amount of toluene in the mobile phases.

Concluding remarks

The behavior of hydrophobic fullerene compounds, C60,C70, C60 pyrr and C60-COOH, in non-aqueous capillary electrophoresis was studied in depth. The use of a long chain alkyl ammonium salt (TDAB) at a high concentration to generate a fullerene-TDAB charged complex and a short chain alkyl ammonium salt (TEAB) at a lower concentration to reduce EOF was needed in order to achieve good electrophoretic performance for all fullerenes studied.. The organic solvent composition of the BGE had a significant effect on resolutions and migration times of the fullerenes.

It was observed that acetic acid is needed in order to obtain the separation of the studied compounds. The migration times of the compounds increased considerably with acetic acid content due to its high viscosity. The addition of MeOH to the BGE shortened the analysis time by reducing the migration time of C60-COOH because of interactions between MeOH and the functional group of this compound.

Acceptable instrumental limits of detection at ppm level (as expected with this kind of methodology) were obtained, with good run-to-run and day-to-day precisions at two concentration levels and RSD values lower than 15%, which made it possible to use the method to analyze these compounds in samples with a high enough concentration. The developed method was applied to the determination of C60 in a commercial cosmetic cream. The results obtained in this work showed that NACE is an inexpensive, and low solvent consumption method, that can be proposed as alternative to conventional LC for the analysis of C60 in commercial cosmeticcreams.

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References

1. Jafvert CT, Kulkarni PP (2008) Buckminsterfullerene's (C60) Octanol-Water Partition Coefficient (Kow) and Aqueous Solubility. Environ Sci Technol 42:5945-5950

2. Pycke BFG, Benn TM, Herckes P, Westerhoff P, Halden RU (2011) Strategies for quantifying C60 fullerenes in environmental and biological samples and implications for studies in environmental health and ecotoxicology. TrAC, Trends Anal Chem 30:44-57

3. Murayama H, Tomonoh S, Alford JM, Karpuk ME (2004) Fullerene production in tons and more: from science to industry. Fullerenes, Nanotubes, Carbon Nanostruct 12:1-9

4. Maralikova B, Weinmann W (2004) Confirmatory analysis for drugs of abuse in plasma and urine by high-performance liquid chromatography-tandem mass spectrometry with respect to criteria for compound identification. J Chromatogr B: Anal Technol Biomed Life Sci 811:21-30

5. Isaacson CW, Kleber M, Field JA (2009) Quantitative analysis of fullerene nanomaterials in environmental systems: a critical review. Environ Sci Technol 43:6463-6474

6. Farre M, Perez S, Gajda-Schrantz K, Osorio V, Kantiani L, Ginebreda A, Barcelo D (2010) First determination of C60 and C70 fullerenes and N-methylfulleropyrrolidine C60 on the suspended material of wastewater effluents by liquid chromatography hybrid quadrupole linear ion trap tandem mass spectrometry. J Hydrol 383:44-51

7. Bouchard D, Ma X (2008) Extraction and high-performance liquid chromatographic analysis of C60, C70, and [6,6]-phenyl C61-butyric acid methyl ester in synthetic and natural waters. J Chromatogr A 1203:153-159

8. Moussa F, Pressac M, Genin E, Roux S, Trivin F, Rassat A, Ceolin R, Szwarc H (1997) Quantitative analysis of C60 fullerene in blood and tissues by high-performance liquid chromatography with photodiode-array and mass spectrometric detection. J Chromatogr B: Biomed Sci Appl 696:153-159

9. Xia XR, Monteiro-Riviere NA, Riviere JE (2006) Trace analysis of fullerenes in biological samples by simplified liquid-liquid extraction and high-performance liquid chromatography. J Chromatogr A 1129:216-222

10. Chae SR, Hotze EM, Xiao Y, Rose J, Wiesner MR (2010) Comparison of methods for fullerene detection and measurements of reactive oxygen production in cosmetic products. Environ Eng Sci 27:797-804

11. Benn TM, Westerhoff P, Herckes P (2011) Detection of fullerenes (C60 and C70) in commercial cosmetics. Environ Pollut 159:1334-1342

12. Cottet H, Simo C, Vayaboury W, Cifuentes A (2005) Nonaqueous and aqueous capillary electrophoresis of synthetic polymers. J Chromatogr A 1068:59-73

13. Tamisier-Karolak SL, Pagliarusco S, Herrenknecht C, Brettreich M, Hirsch A, Ceolin R, Bensasson RV, Szwarc H, Moussa F (2001) Electrophoretic behavior of a highly water-soluble dendro[60]fullerene. Electrophoresis 22:4341-4346

14. Cerar J, Pompe M, Gucek M, Cerkovnik J, Skerjanc J (2007) Analysis of sample of highly water-soluble Th-symmetric fullerenehexamalonic acid C66(COOH)12 by ion-chromatography and capillary electrophoresis. J Chromatogr A 1169:86-94

15. Chan KC, Patri AK, Veenstra TD, McNeil SE, Issaq HJ (2007) Analysis of fullerene-based nanomaterial in serum matrix by CE. Electrophoresis 28:1518-1524

16. Treubig JM, Brown PR (2000) Novel approach to the analysis and use of fullerenes in capillary electrophoresis. J Chromatogr A 873:257-267

17. Su HL, Kao WC, Lee Cy, Chuang SC, Hsieh YZ (2010) Separation of open-cage fullerenes using nonaqueous capillary electrophoresis. J Chromatogr A 1217:4471-4475

18. Wan TSM, Leung GNW, Tso TSC, Komatsu K, Murata Y (1995) Non-aqueous capillary electrophoresis as a new method for the separation of fullerenes. Proc - Electrochem Soc 95-10:1474-1487

19. Núñez ON, Astefanei A, Gallart-Ayala H, Moyano E, Galceran MT (2011) Analysis of fullerenes (C60 to C84) and C60-fullerene derivatives by liquid chromatography-atmospheric pressure photoionization/mass spectrometry. Oral communication, 13th Instrumental Analysis Conference, 14th to 16th November, Barcelona, Spain.

20. Takayanagi T, Wada E, Motomizu S (1997) Electrophoretic mobility study of ion association between aromatic anions and quaternary ammonium ions in aqueous solution. Analyst 122:57-62

21. Wang H, Hu H, Ding T, Gu J, Dai R, Fu R (1998) Study on the enantiomeric separation of adrenalines by capillary zone electrophoresis. Sepu 16:22-25

Figure captions

Figure 1. Fullerene and C60 fullerene derivatives chemical structures.

Figure 2. (a) Electropherograms of a mixture of fullerenes (100 mg/L) at different TDAB concentrations (from 90 to 250 mM). Other BGE conditions: 50 mM TEAB, 6% MeOH and 10% acetic acid in acetonitrile:chlorobenzene 1:1 (*v/v*). Acquisition conditions: capillary voltage: +30 kV; hydrodynamic injection: 5 s (13.5 kPa); wavelength: λ 350 nm. Peak identification: 1, C60; 2, C70; 3, C60 pyrr; 4, C60-COOH.

Figure 3. a) Separation of fullerenes by NACE under optimum conditions. b) Electropherogram obtained for a commercial cosmetic cream and the same product fortified with 5 mg/L of C60. c) LC-MS chromatogram of C60 in a commercial cosmetic cream BGE: 200 mM TDAB, 40 mM TEAB, 6% MeOH and 10% acetic acid in acetonitrile:chlorobenzene 1:1 (*v/v*). Acquisition conditions: capillary voltage: +30 kV; hydrodynamic injection: 5 s (13.5 kPa); acquisition wavelength: λ 350 nm. LC-MS conditions as indicated in experimental section Peak identification: 1, C60; 2, C70; 3, C60 pyrr; 4, C60-COOH.

Table 1. Effect of TDAB concentration on the separation of the studied fullerenes

TDAB (mM)	Height				Rs	
	C70	C60	C60 pyrr	C60-COOH	C70-C60	C60 pyrr-C60-COOH
90	1373	3936	2220	2601	0,9	0,6
100	1400	3980	2409	2056	1,1	2,6
150	1471	8132	2737	2340	1,2	4,5
200	1625	10858	2820	2573	1,2	5,1
250	1502	8058	2076	1823	1,1	5,9

Table 2. Effect of TEAB concentration on the apparent mobilities of the studied fullerenes

TEAB (mM)	μ_{app} x 10^4 (cm^2/Vs)			
	C70	C60	C60 pyrr	C60-COOH
0	1.75	1.75	1.5	1.5
20	1.22	1.2	1.02	1.01
40	1.0	0.98	0.76	0.72
50	0.96	0.94	0.68	0.65
60	0.9	0.89	0.62	0.59

Table 3. Effect of acetic acid and methanol content in the BGE on the separation of the studied fullerenes

Acetic acid (%)[a]	t_m (min)				Rs	
	C70	C60	C60 pyrr	C60-COOH	C70-C60	C60 pyrr-C60-COOH
6	14.9	15.0	17.9	22.8	0,7	24
10	16.6	16.9	22.3	25.0	1,2	13
16	19.3	19.6	27.4	28.1	1,3	2,8
20	23.1	23.5	34.3	34.3	1,3	0
MeOH (%)[b]	t_m (min)				Rs	
	C70	C60	C60 pyrr	C60-COOH	C70-C60	C60 pyrr-C60-COOH
0	16.6	16.9	22.3	25.0	1.2	13
4	16.7	17.0	22.2	24.0	1.2	8.6
6	16.7	17.0	22.2	23.3	1.2	5.1
8	16.7	17.0	22.1	22.2	1.2	0.9

[a] other BGE conditions: 200 mM TDAB, 40 mM TEAB in acetonitrile-chlorobenzene 1:1 (*v/v*)

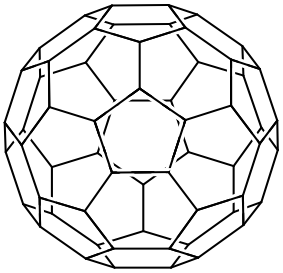
[b] other BGE conditions: 200 mM TDAB, 40 mM TEAB and 10% acetic acid in acetonitrile-chlorobenzene 1:1 (*v/v*)

Table 4. Instrumental quality parameters

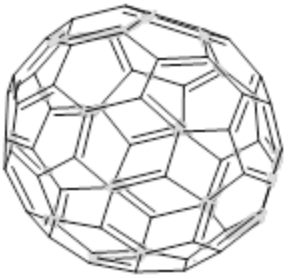
	LODs (mg/L)	LOQs (mg/L)	Run to run precision, % RSD (n=5)			Day-to-day precision, % RSD (n=5x3)		
			Migration time	Conc. (low level)[a]	Conc. (medium level)[b]	Migration time	Conc. (low level)[a]	Conc. (medium level)[b]
C70	3.7	11.1	0.4	6.2	3.7	1.2	8.1	6.3
C60	1.0	3.0	0.3	5.8	2.5	1.3	10.1	5.8
C60 pyrr	1.3	3.9	1.0	8.0	1.5	1.2	14.5	4.1
C60 COOH	2.8	8.4	0.5	5.7	1.7	1.6	10.1	1.9

[a] LOQ
[b] 50 mg/L

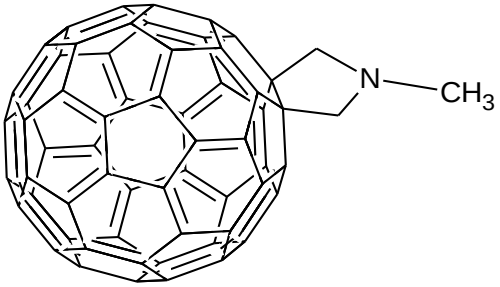
Figure 1



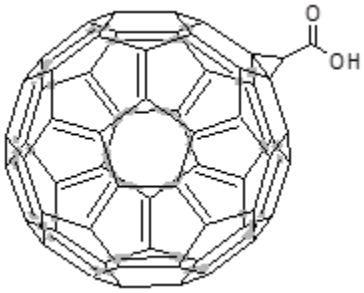
Fullerene C60 (C60)
(1)



Fullerene C70 (C70)
(2)



N-methyl fulleropyrolidine (C60 pyrr)
(3)



1,2-methanofullerene-C60-61-carboxylic acid (C60-COOH)
(4)

Figure 2

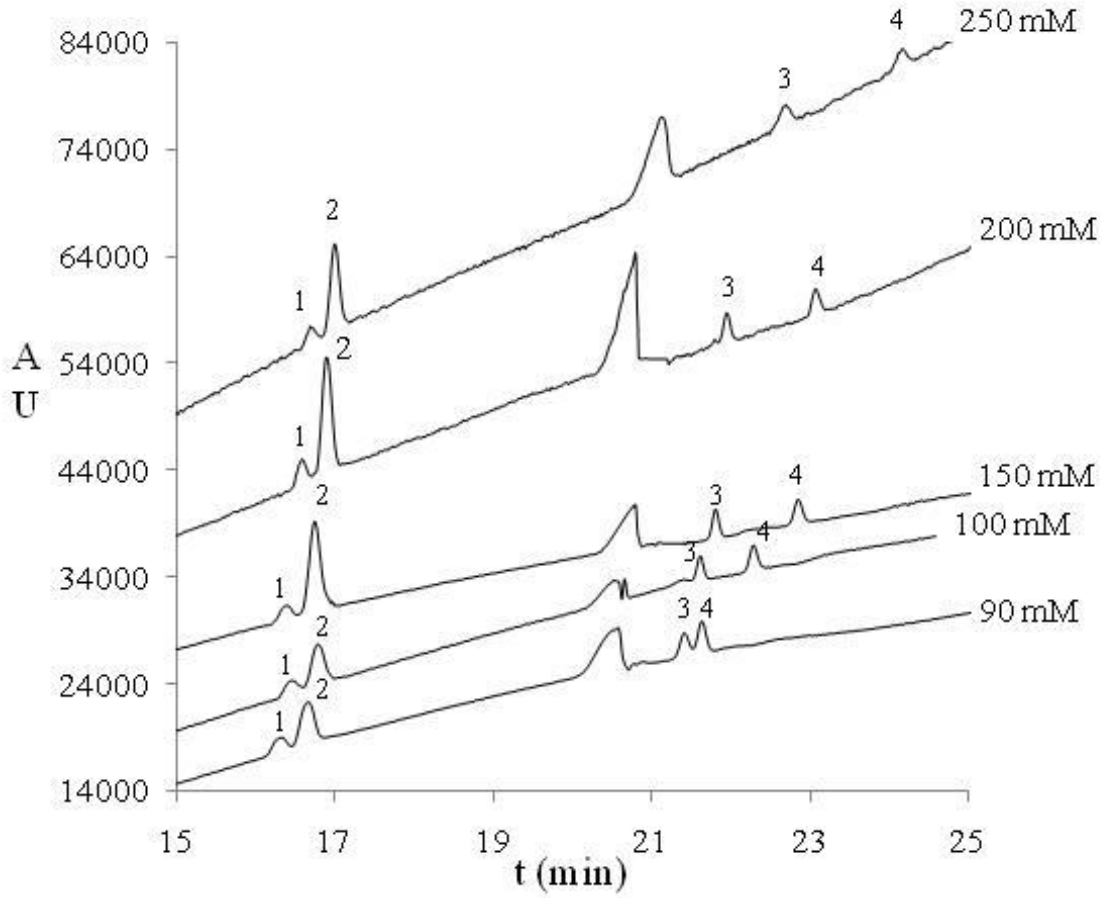
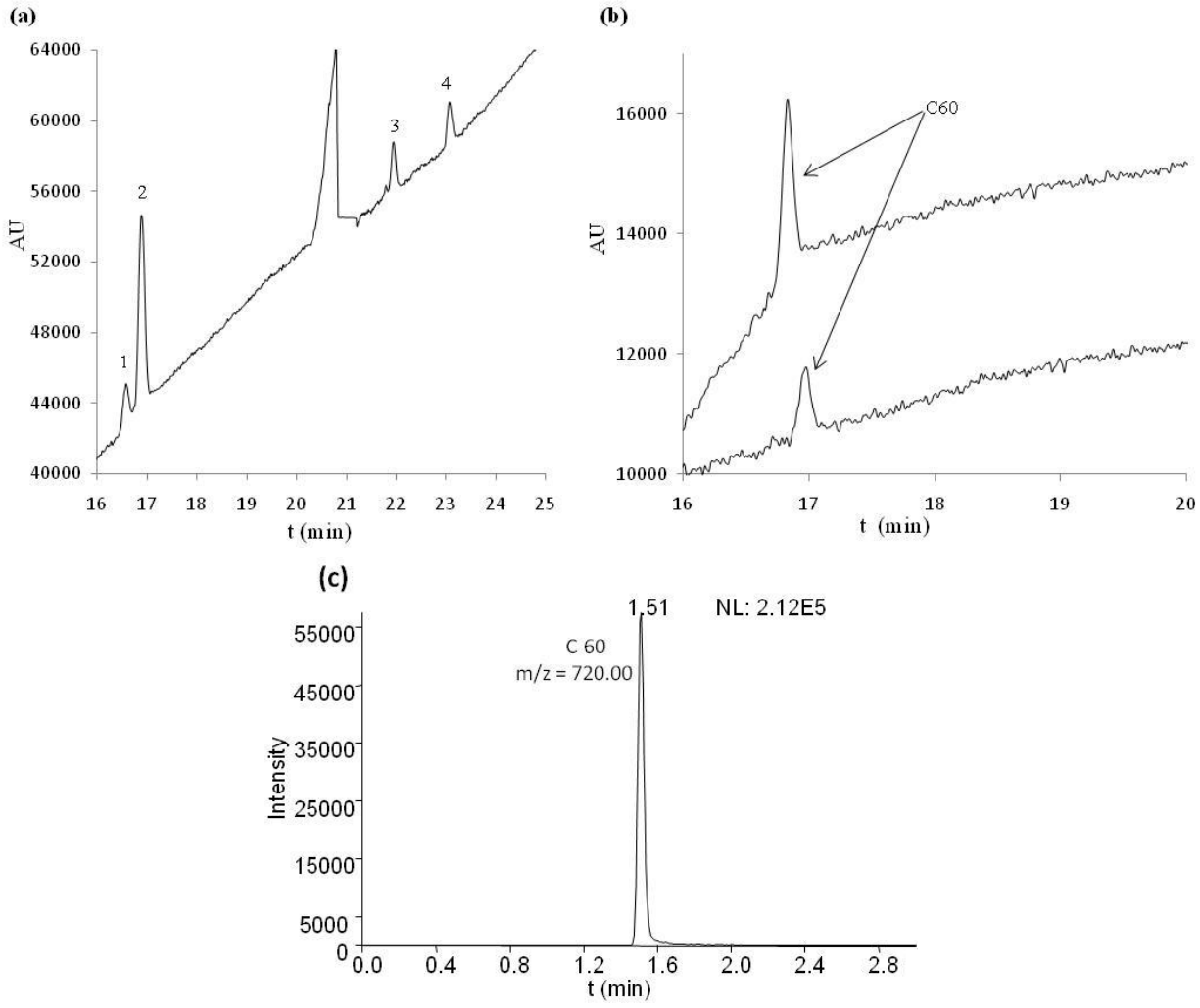

84000
74000
64000
54000
44000
34000
24000
14000
AU
15
17
19
21
23
25
t (min)
250 mM
200 mM
150 mM
100 mM
90 mM
1
2
3
4

Figure 3


(a)
AU
t (min)
1
2
3
4
(b)
AU
t (min)
C60
(c)
Intensity
t (min)
1.51
NL: 2.12E5
C 60
m/z = 720.00