J Incl Phenom Macrocycl Chem (2009) 64:83–94
DOI 10.1007/s10847-009-9539-4

ORIGINAL ARTICLE

Inclusion complexes of γ-cyclodextrin and carboxyl-modified γ-cyclodextrin with C_{60}: synthesis, characterization and controlled release application via microgels

Fifere Adrian · Tania Budtova · Elena Tarabukina · Mariana Pinteala · Spulber Mariana · Cristian Peptu · Valeria Harabagiu · Bogdan C. Simionescu

Received: 10 September 2008 / Accepted: 7 January 2009 / Published online: 23 January 2009

Abstract Carboxyl modified γ-cyclodextrin (CDSA) with a substitution degree of about 9.5 was prepared by the esterification of γ-cyclodextrin (CD) with succinic anhydride in pyridine at 90 °C. The chemical composition and the structure of CDSA were characterized by FT-IR, MALDI-TOF, X-ray diffraction pattern, potentiometric titration and TGA. Modified and native γ-cyclodextrin associate with fullerene (C_{60}) in DMF-toluene mixture resulting 1:1 CDSA:C_{60} and CD:C_{60} inclusion complexes. Aqueous solutions of native cyclodextrin, carboxyl-modified cyclodextrin and their inclusion complexes with C_{60} were used as microgel solvent (or swelling agent) for controlled release application. The release of solutions was induced by shear stress and demonstrated using rheo-optical set-up.

Keywords Carboxyester modified γ-cyclodextrin · Fullerene C_{60} · Inclusion complexes · Shear induced release from microgel

F. Adrian · M. Pinteala · S. Mariana (✉) · C. Peptu · V. Harabagiu · B. C. Simionescu
Petru Poni Institute of Macromolecular Chemistry, 700487 Iasi, Romania
e-mail: mari_19ian@yahoo.com

T. Budtova
Mines ParisTech, Centre de Mise en Forme des Matériaux (CEMEF), 06904 Sophia-Antipolis, France

E. Tarabukina
Institute of Macromolecular Compounds, Russian Academy of Sciences, 199004 St. Petersburg, Russia

Introduction

Making fullerenes "soluble" in water is of great interest for their specific biological applications like enzyme inhibition, antiviral activity, DNA cleavage, photodynamic therapy, electron transfer, etc. [1]. As already known, the large spherical fullerene C_{60} can be reasonably well dissolved in non-polar solvents, such as toluene and dichloromethane, is moderately soluble in nonpolar, aprotic and polar, nonaqueous solvents, but is totally insoluble in water. Until recently, the solubilisation of C_{60} in aqueous solution was possible only by chemical derivatisation of fullerene, which leads to a partial loss of its aromatic character [2]. One example of fullerene derivative was described in 1993 by Schinazi et al. [3], which tested carboxyl-modified fullerene for antiviral activity in cells acutely and chronically infected with HIV-1 and in cell free systems, suggesting that carboxylic water-soluble fullerene derivatives have potential selective activity against HIV-1.

The most recent and successful strategy to improve the water-solubility of C_{60} is to surround it with water-soluble host molecules such as calixarenes [4–6], cyclodextrins [7–9] or to incorporate C_{60} in polymeric structures [10]. The main advantage of using non-covalent linkages is the fact that the electronic structure of fullerene remains only slightly changed as compared to the structure of derivatized fullerene. In particular, this difference was shown for pairs of charge transfer complexes of poly(N-vinylpyrrolidone) (PVP) with fullerene C_{60} (PVP/C_{60}) or with modified C_{60} (PVP/modified C_{60}) [11]. That is why unique properties of fullerene caused by its structure can be revealed full-bodily if it is complexed with polymers.

Cyclodextrins (CDs) are cyclic oligosaccharides having a hydrophilic external surface which confers aqueous

solubility, and a relatively hydrophobic inner cavity able to incorporate molecules of appropriate size. β- and γ-CD have been reported to form inclusion complexes with C_{60}. Because the dimension of CD cavity (0.57 nm for β-CD; 0.95 nm for γ-CD) is smaller than the size of C_{60} (1.00 nm), a full accommodation of C_{60} inside CD is not possible. Thus, theoretically two CD molecules are needed to bound one C_{60}, i.e., a 2:1 β- or γ-CD:C_{60} complex is formed [12, 13]. Even a full accommodation of C_{60}, 1:1 inclusion complexes between cyclodextrins and C_{60}, as described in this paper, have already described in the literature [14–17]. To use the unique properties of CDs as host macrocycles for inclusion complexation, a wide variety of their chemically modified derivatives have been prepared [18, 19].

The goal of our investigation is to prepare and characterize fullerene/γ-cyclodextrin complexes, with native and chemically modified γ-CDs and to demonstrate one option of their controlled delivery. The cyclodextrin was esterified with succinic anhydride leading to the formation of a macrocycle functionalized with carboxyl groups (CDSA). CD/C_{60} and CDSA/C_{60} complexes were dissolved in water and used as a solvent for a synthetic superabsorbent microgel, the latter being a model carrier. Solvent release from the gel was induced by shear stress and demonstrated with rheo-optical tool using the same principle as described in literature [20].

Materials and methods

Materials

γ-Cyclodextrin (CD) (Aldrich) was dried at low pressure and 80 °C for 48 h. Pyridine was dried by distillation on potassium hydroxide. Fullerene-C_{60} (C_{60}) was purchased from MER Corporation and used without further purification. All other reagents and solvents, succinic anhydride (SA), toluene and dimethylformamide (DMF) were from Aldrich and used as received.

Aqua Keep 10 SHNF hydrogel (AK) was kindly provided by Kobo Products Inc. It is composed of 75–25% poly(sodium acrylate-*co*-acrylic acid) chemically cross-linked with N,N′-methylenebisacrylamide. The initial state of the gel was a powder of dry spherical particles with diameter of 5–60 μm.

A silicone oil, polydimethylsiloxane (PDMS) Rhodorsil 47 V 200000 (Rhodia, France) with viscosity of 230 Pa s, was selected as suspending matrix for swollen microgels. Silicone oil was chosen because it is transparent, chemically inert with respect to swollen gels and the solvents used and sufficiently viscous, enabling to exert the stresses necessary to deform the gel particles.

Methods

Complex characterization

FT-IR characterisation was performed on a Bruker Vertex 70, instrument using KBr disc method. NMR spectra were obtained on a DRX 400 Avance Bruker 400 MHz spectrometer. UV–vis absorptions were measured on an Analytik Specord 200 Jena spectrophotometer. MALDI-TOF mass spectrum of CDSA was recorded on a Reflex IV (Bruker Saxonia) instrument. The samples were prepared by mixing the compound solution (10 mg/mL) in a 1:1 v/v acetonitrile:water mixture, with a saturated solution of α-cyano-4-hydroxycinnamic acid matrix in the same solvent mixture in a volumetric ratio of 1/100. Then, 1 μL of the resulting mixture was placed onto the sample plate and the solvent was evaporated at room temperature. Spectra were recorded in positive linear mode. All experiments were carried out collecting 200 laser shots with laser power slightly above threshold to obtain a clear signal. The calibration was performed using a Bruker peptide mixture for the 1,000–3,000 Da *m/z* span. TGA analyses were performed in oxygen with a temperature scanning rate of 10 K/min on a Q-1500 D MOM Budapest system. The wide angle X-ray diffraction (WAXD) patterns were recorded on a Bruker AXS D8 advance X-ray diffractometer with scanning scope of 0°–40°, scanning speed of 4°/min, using Cu Kα radiation.

Titration of carboxylic groups in CDSA and CDSA/C_{60} complex

The potentiometric titration was done with a TitrolineAlpha Plus apparatus. The aqueous solution of 0.003 g/L of CDSA or CDSA/C_{60} complex was titrated with 10^{-2} M aqueous sodium hydroxide. The equivalence points were calculated as an average of three measurements and the molar content of carboxylic units was calculated with the relation (1):

$$N_{\mathrm{COOH}} = \frac{M_{\mathrm{CD}}}{\frac{V_{\mathrm{a}}}{V_{\mathrm{b}}} \cdot \frac{C_{\mathrm{CDSA}}}{C_{\mathrm{NaOH}}} M_{\mathrm{NaOH}} - M_{\mathrm{SA}}} \quad (1)$$

where N_{COOH} is the average molar number of carboxylic groups in CDSA; M_{NaOH}, M_{CD} and M_{SA} are the molar masses of sodium hydroxide, unmodified cyclodextrin and succinic anhydride, respectively; V_{a} and V_{b} are the volume of CDSA and of sodium hydroxide solution at equivalence point, respectively; C_{CDSA} and C_{NaOH} are the concentrations of CDSA and NaOH solutions in g/L. An average molar content of 9.6 carboxylic groups was obtained for CDSA.

From the average number of carboxylic groups on each CDSA macrocycle, the molar ratio between components in CDSA/C_{60} complex of about 1:1 was calculated with the relation (2).

$$\frac{\text{CDSA}}{\text{C}_{60}}(\text{molar}) = \frac{M_{\text{C}_{60}}}{N_{\text{COOH}}\frac{V_a}{V_b}\cdot\frac{C_{\text{CDSA/C}_{60}}}{C_{\text{NaOH}}}M_{\text{NaOH}} - M_{\text{SA}}} \quad (2)$$

where M_{C60} is the molecular weight of C_{60} and $C_{CDSA/C60}$ is the concentration of CDSA/C_{60} complex in g/L.

Determination of microgel swelling degree

In order to determine gel swelling degree at equilibrium, aqueous solutions of CD, CDSA, CD/C_{60} or CDSA/C_{60} of different concentrations (from 0 to 1 g/dL for CD and CDSA and from 0 to 0.34 g/dL for CD/C_{60} and CDSA/C_{60} complexes) were added drop by drop to precisely weighted dry gel particles until the suspension becomes fluid due to the presence of the excess of solvent. At each step of solvent addition, the suspension was let to relax after absorbing the solvent. The weight of solvent added just before the excess of solvent appeared was noted and the degree of swelling at equilibrium Q was calculated as the ratio between the weight of absorbed solvent and the weight of the dry particles.

Rheo-optics

For the rheo-optical experiments, microgel was swollen up to 70% of the equilibrium value by simply adding a certain amount of solution to the dry particles, which leads to complete solution absorption by the gel. With this procedure the solution concentration inside the gel is the same as in the prepared one.

Rheo-optical set-up was used to observe the shear-induced release of the aqueous solutions of CD, CDSA, CD/C_{60} and CDSA/C_{60} from a swollen microgel, as described in [21] for model systems. A simple shear flow was generated by a counter rotating plate–plate system [20, 22, 23]. Two plates are transparent and rotate in opposite directions. An optical microscope placed above the upper rotating plate allows observations in the plane formed by the flow and vorticity directions. All the experiments were recorded by a CCD camera coupled to the image acquisition complex. The gap between the plates was 0.7 mm. By adjusting the relative velocities of the plates, a selected particle immersed in the PDMS can be immobilized in the laboratory framework and its behaviour can be monitored. The shear rate was varied between 0.5 and 25 s^{-1}. All the experiments were performed at room temperature. A detailed description of sample preparation and experimental procedure is given in literature [23].

The initial swollen gel is spherical. Under shear, the gel deforms, solvent is released and can be detached [20, 23]. After stopping the shear, gel recovers its initial spherical shape. In order to determine gel volume loss, gel diameter before and after shearing was measured and the relative volume loss ΔV was calculated as $\Delta V = (V_0 - V(t))/V_0$, where $V(t)$ and V_0 are gel volume at a moment t and $t = 0$, respectively. The experiments were performed in the following way: a selected particle was subjected to a constant shear rate during a certain time (from 10 s to 2 min), then shear was stopped and the diameter of the relaxed gel was measured. In subsequent runs, the particle was submitted to the increasing shear rates by increasing the rotation velocities of the plates. The gel diameter was measured by analysing the recorded tapes with the help of image analysis software Visilog from Noesis 2000. The experimental errors of rheo-optical measurements (shear rate, dimensions of objects) were smaller than 10%.

Results and discussion

Synthesis

Synthesis of CDSA

CDSA was prepared by the esterification of CD with SA in pyridine at 90 °C, according to a procedure proposed by Gonzalez and Rossi [21] for the chemical modification of β-CD with SA. The procedure was as follows. Into a three necked round bottomed vessel, equipped with magnetic stirrer, reflux condenser and argon inlet, a solution of 5.34 g (53.4 mmol) SA in 72 mL anhydrous pyridine was added under stirring over a solution of 6.00 g (4.6 mmol) CD in 100 mL anhydrous pyridine at 60 °C. The mixed solutions were heated at 90–100 °C under stirring and the obtained opalescent solution was diluted with 40 mL anhydrous pyridine. The reaction was continued at this temperature for 9 h under stirring in argon atmosphere, and then the solvent was evaporated in vacuum. The solid was washed several times with diethyl ether to remove all traces of pyridine and suspended in acetone under stirring overnight to remove the unreacted anhydride. The purified CDSA was obtained as a white solid in about 90% yield.

Synthesis of the CD/C_{60} complex (Scheme 1)

Into a three necked round bottomed flask provided with reflux condenser, magnetic stirrer, argon inlet and dropping funnel, a solution of 201 mg (0.155 mmol) CD in 30 mL water was prepared and heated at 100 °C. A solution of 60 mg (0.083 mmol) C_{60} in 30 mL toluene was added drop wise through the dropping funnel and the heterogeneous mixture was maintained under strong stirring and inert atmosphere at 100–107 °C for 3 days. After cooling up the mixture, three liquid phases (water uncoloured phase, emulsion violet phase and toluene dark violet phase) and a violet precipitate were obtained. The precipitate, the

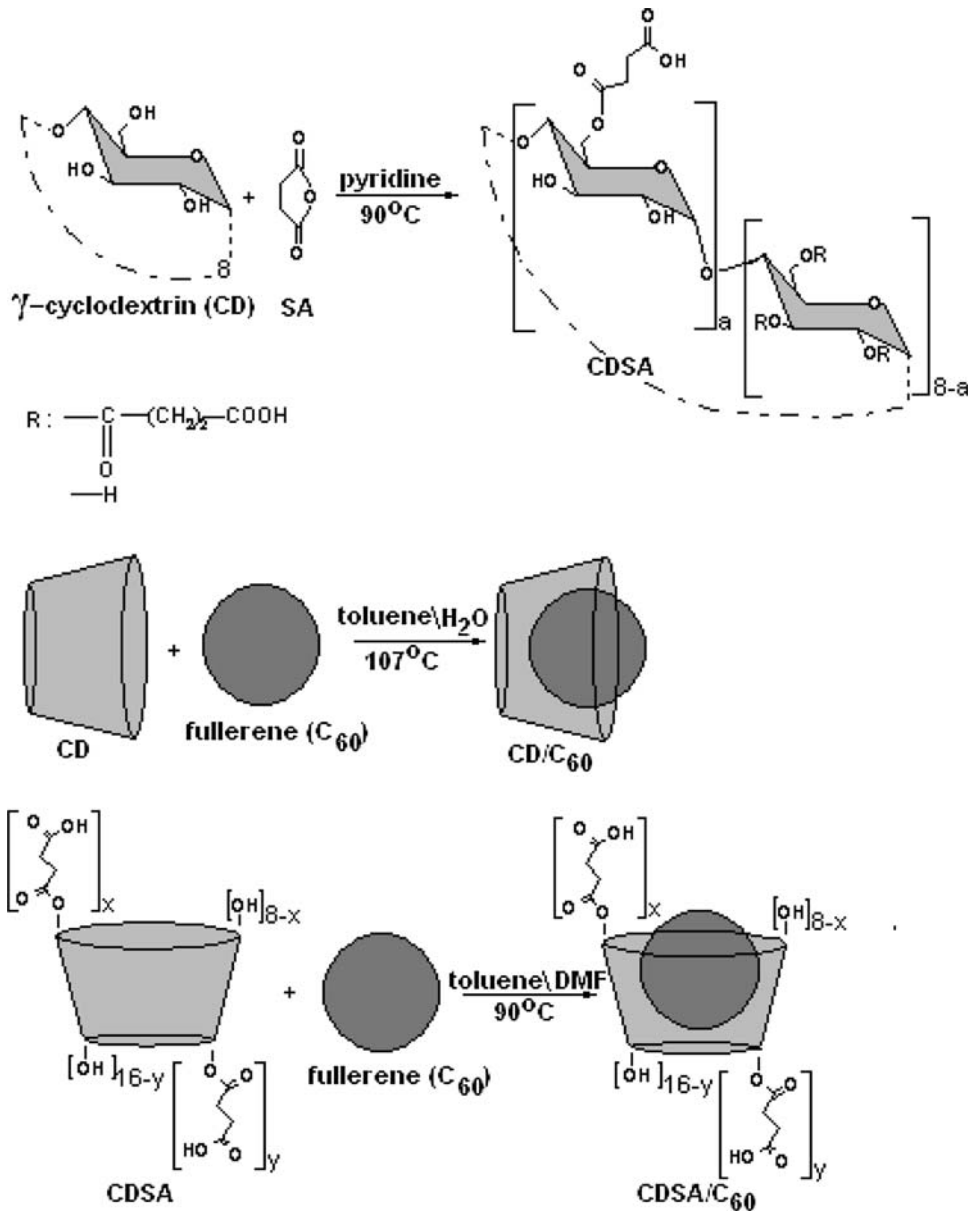
aqueous and emulsion phases were mixed, diluted with 20 mL water and centrifuged together to completely separate the complex. The obtained complex was dried, washed firstly with toluene until no coloration of the solvent was observed and secondly with cold water to separate the uncomplexed C_{60} and CD, respectively. After drying, 64.4 mg of violet solid complex were obtained.

Synthesis of the CDSA/C_{60} complex (Scheme 1)

Into a three necked round bottomed flask, provided with reflux condenser, magnetic stirrer, argon inlet and dropping funnel, a solution of 250 mg (0.114 mmol) CDSA in 23 mL DMF was introduced. A solution of 40 mg (0.057 mmol) C_{60} in 23 mL toluene was added drop wise through the dropping funnel. The mixture was stirred at room temperature for 10 min and then at 90 °C for 48 h. The solvents were then evaporated under vacuum and the obtained solid was washed with large amounts of toluene until no C_{60} was observed in toluene phase. The brown solid product was washed with diethyl ether to remove solvent traces and three times with small amounts of ethyl alcohol to remove the unreacted CDSA. About 88.8 mg of brown solid complex were obtained after purification.

Characterization of CDSA and of cyclodextrin/C_{60} complexes

The synthesis of modified cyclodextrin and of CD/C_{60} and CDAS/C_{60} complexes is described in Methods Section and



Scheme 1 Synthesis of CDSA, CD/C_{60} and CDSA/C_{60} complexes

schematically presented in Scheme 1. All new compounds were extensively characterized using FT-IR, NMR, UV-vis, TGA, potentiometric titration and mass spectrometry.

FT-IR absorption spectra

The FT-IR absorption spectra of the initial γ-cyclodextrin, CDAS and their complexes with C_{60} are presented in Fig. 1, from a to d, respectively. The modification of CD is clearly demonstrated by the presence of the carbonyl ester high band at 1733 in CDSA spectrum (Fig. 1b). Moreover, some CD specific vibrations were diminished (708 cm^{-1}) and others were shifted with 1 to 7 cm^{-1} through its asymmetrical esterification. As previously showed by Boulas et al. [24], the complexation of CD with C_{60} determines minimal shifts (less than 6 cm^{-1}) of the ring vibrations (583, 708, 758 and 943 cm^{-1}), of the coupled C–O–C stretching/O–H bending vibrations (1158 cm^{-1}), and of the coupled C–O/C–C stretching/O–H bending vibrations (1027 and 1080 cm^{-1}). Larger shift, about 9 cm^{-1} was registered for the coupled C–H/O–H bending vibrations at 1416 cm^{-1}, which may indicate the interactions between CD inner cavity and C_{60} (Fig. 1a, c). Contrary to CD/C_{60}, the CDSA/C_{60} complex showed higher shifts of 6–8 cm^{-1} for some of the cyclodextrin ring vibrations (586 and 946 cm^{-1}) (Fig. 1b, d).

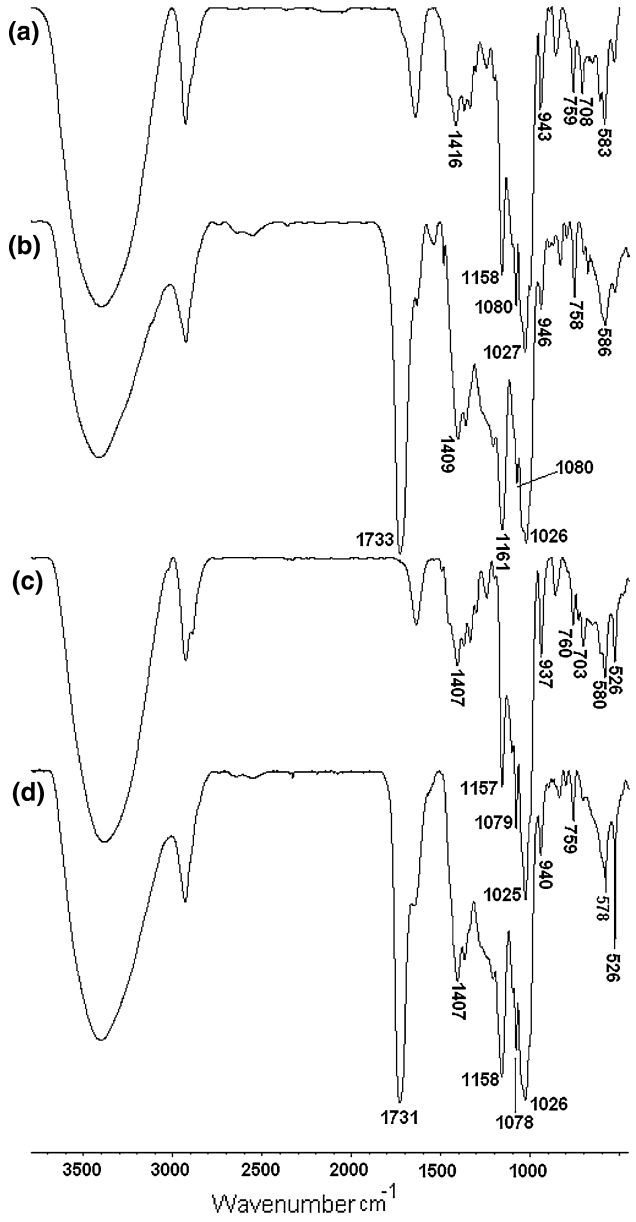


Fig. 1 FT-IR spectra of CD (**a**), CDSA (**b**), CD/C_{60} complex (**c**), CDSA/C_{60} complex (**d**)

The FT-IR spectra of C_{60} complexes with both CD and CDSA (Fig. 1c, d) show that cyclodextrin absorptions are practically covering fullerene bands. Only the stronger band at 526 cm^{-1}, visible in FT-IR spectra of both complexes, can be clearly attributed to C_{60} [24].

^{1}H-NMR and ^{13}C-NMR characterization

A deeper structural analysis was performed by ^{1}H- (Figs. 2, 3, 4) and ^{13}C-NMR (Figs. 5, 6) in D_2O and in DMSO-d_6. Figures 2 and 3 compare the ^{1}H-NMR resonance peaks of unmodified and modified CDs in D_2O and DMSO-d_6, respectively. As previously shown by Schneider et al. [25], the chemical shifts of different protons belonging to CD ring are not identical in these two solvents due to substantial differences in the populations around the C^5–C^6 bond and in the rate of exchange of OH protons (much faster for D_2O as compared to DMSO-d_6). The position of resonance peaks is also dependent on the acquisition temperature (24.2 °C in our experiments).

As one can see from Figs. 2 and 3, the anomeric protons appearing as unique doublet signals in non-modified CD at 5.10 ppm in D_2O (Fig. 2a) and 4.89 in DMSO-d_6 (Fig. 3a) are splitted into two signals in modified CD, denoting an asymmetric substitution of CD ring. In D_2O (Fig. 2b), the anomeric signals are larger and centred at 5.10 and 5.30 ppm. In DMSO-d_6 (Fig. 3b), a first peak remain unchanged at 4.89 ppm and the second one is larger between 5.10 and 5.12 ppm. Moreover, following the esterification of the sixth position, the peaks corresponding to the H^6 protons in the spectra of CDSA are enlarged and downfield shifted as compared to CD. The peaks of H^a and H^b methylene protons linked to ester and carboxylic groups, respectively, in the spectrum of CDSA performed in DMSO-d_6 are masked by the solvent (Fig. 3b), but they are identified as quite large signals of two superposed triplets, at 2.70–2.89 ppm in the spectrum performed in D_2O (Fig. 2b) [26].

The ^{1}H-NMR spectra and peak values of CD/C_{60} and of CDSA/C_{60} complexes performed in DMSO-d_6 are

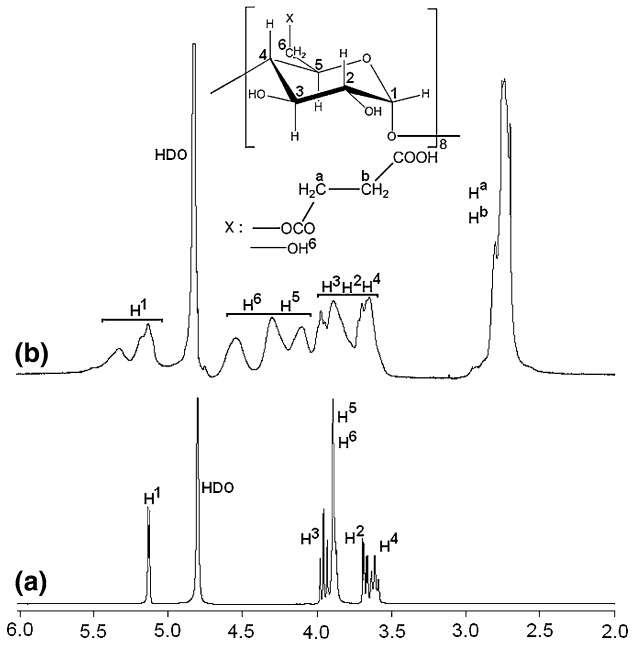


Fig. 2 ^{1}H-NMR spectra of CD (**a**) and CDSA (**b**) in D_2O

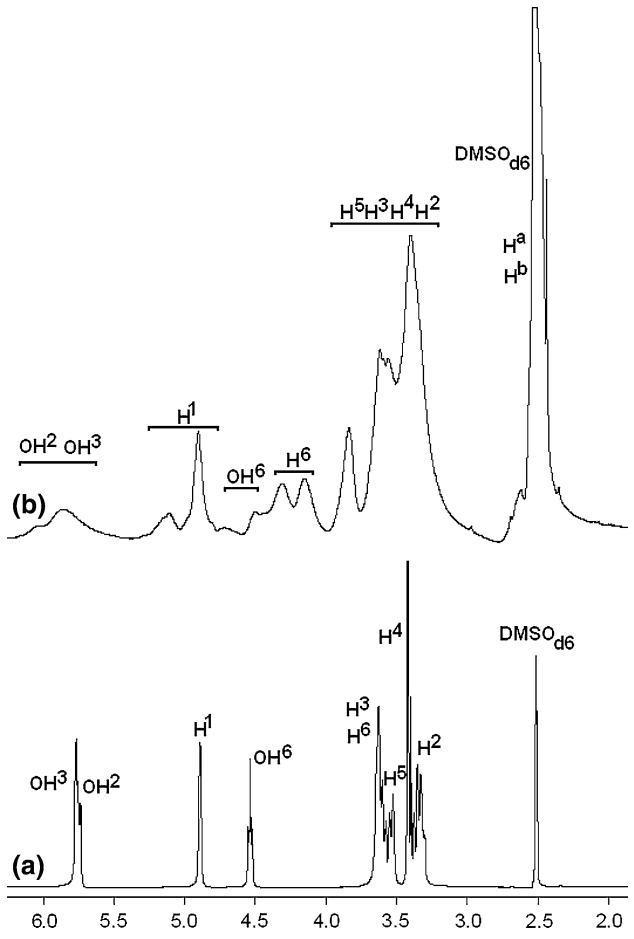


Fig. 3 ^{1}H-NMR spectra of CD (**a**) and CDSA (**b**) in DMSO-d_6

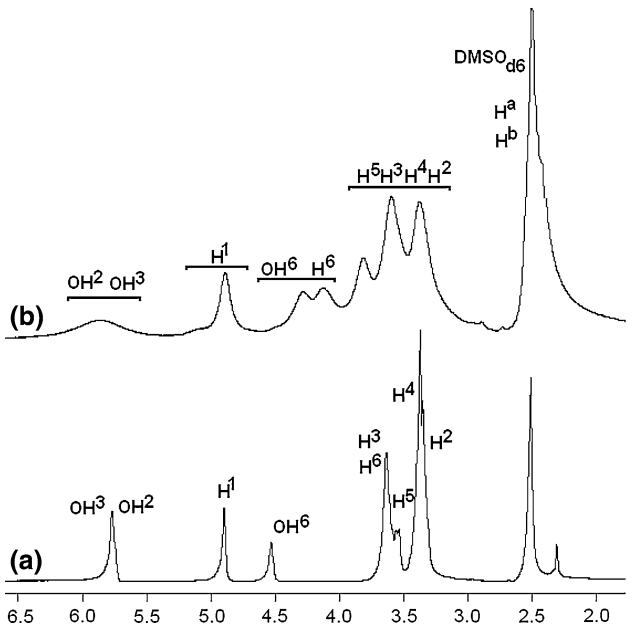


Fig. 4 ^{1}H-NMR spectra of CD/C_{60} complex (**a**) and CDSA/C_{60} complex (**b**) in DMSO-d_6

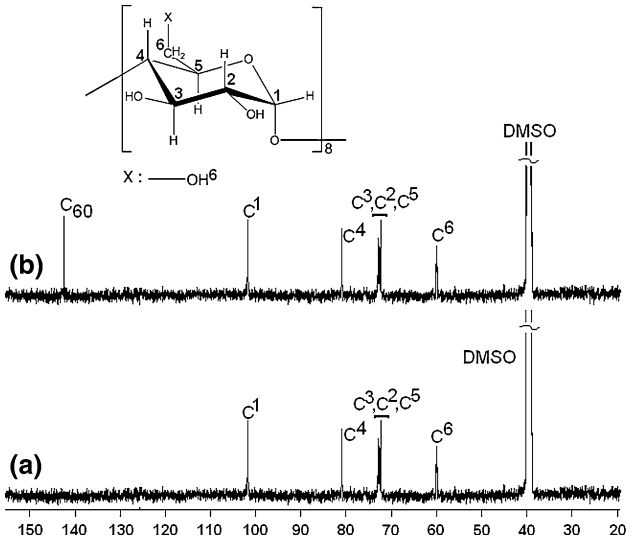


Fig. 5 ^{13}C-NMR of CD (**a**) and CD/C_{60} complex (**b**) in DMSO-d_6

presented in Fig. 4. One can observe that only small differences in the chemical shifts of CD/C_{60} or CDSA/C_{60} as compared to the non complexed corresponding macrocycle molecules (Fig. 3) are evidenced. These differences are mainly due to a conformational change to a more conical structure in the host molecule induced by complexation, as suggested for CD/C_{60} system in [27].

Figures 5 and 6 contain ^{13}C-NMR data of modified and unmodified CDs and of their C_{60} complexes in DMSO-d_6.

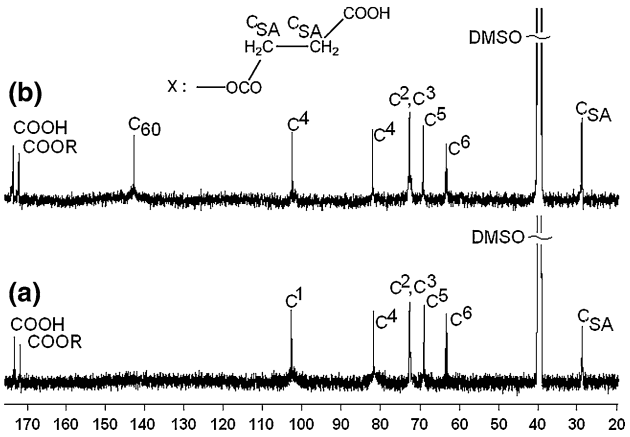


Fig. 6 ^{13}C-NMR spectra of CDSA (**a**) and CDSA/C_{60} complex (**b**) in DMSO-d_6

The ^{13}C-NMR chemical shifts are much better indicators for the structural variation. The observed ^{13}C peak positions of CD (Fig. 5a) and CDSA (Fig. 6a) vary from 60.03 to 63.00 ppm ($\delta = 3$ ppm) for C^6 and from 72.22 to 69.21 ($\delta = 3.01$ ppm) for C^5, showing very few correlation with other constituent induced shifts, as Anderson et al. [27] previously pointed out. The ^{13}C shifts of C^4 reflect syn and anty orientation of the C^6–OR group with respect to the C_5–C_4 bond, taking into consideration the classical chair conformation of glucopyranose units that does not depend on the C^6 substituent [28]. In CDSA spectrum, the resonance peaks corresponding to methylene, ester and carboxyl carbon atoms belonging to SA derived substituent are also visible at 28.58, 172.01 and 173.48 ppm, respectively. As for the CD/C_{60} complex, its ^{13}C-NMR characteristic peaks (Fig. 5b) are slightly upfield shifted ($\delta = -0.06$ to 0.08 ppm) as compared to the initial CD, while for CDSA/C_{60} complex (Fig. 6b) higher downfield and upfield shifts for C^1 and C^2 ($\delta = +0.12 \div +0.31$ ppm) and for C^3 and C^6 ($\delta = -0.14 \div -0.25$ ppm), respectively, were observed when compared to the non-complexed CDSA. The differences in the chemical shifts of cyclodextrins and of their complexes are due to conformational changes of the macrocyclic molecules upon complexation. In addition, ^{13}C-NMR spectra of CD/C_{60} and CDSA/C_{60} complexes show signals at 142.00 and 142.77 ppm, respectively, which were slight upfield shifted from the 143.00 ppm peak of pristine C_{60}. These minor differences mean that CD cavities only slightly disturbed the local magnetic field of C_{60} [25].

Mass spectrometry characterization

The molecular structure of CDSA was also proved by MALDI-TOF analysis (Fig. 7). The resulted peaks are sodium adducts ranging from 1719 to 2519 Da with a mass increment of 100 Da. The *m/z* values of each peak can be calculated by the formula: $X = 1296$ (γ-CD) + $n100$ (SA) + 23 (Na), where n is the molar number of SA units.

The determined degree of functionalization is ranging from 6 to 13. The most abundant product was the CD bearing 10 SA units. The peaks with lower intensity, which are doubling the main series, are K adducts, provided that K ions can be usually found as an impurity in laboratory glassware. Sodium adducts of the carboxylic Na salts formed during the MALDI ionization process can also be observed. The result confirms the average values of 9.5 and

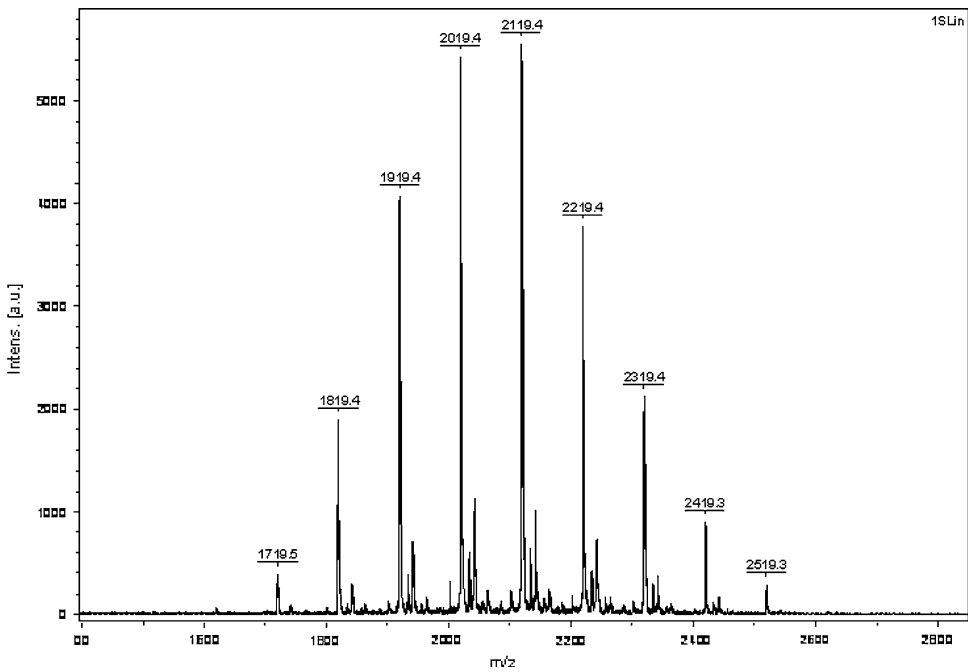


Fig. 7 MALDI-TOF spectra of CDSA

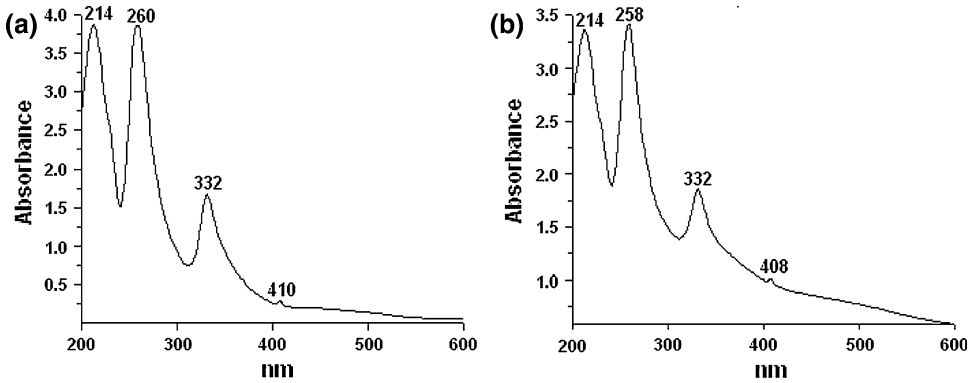


Fig. 8 Electronic absorption spectra of water solutions of (**a**), 0.02 g/dL CD/C_{60} complex; (**b**), 0.03 g/dL CDSA/C_{60} complex

9.6 of the substitution degree of CDSA obtained from the potentiometric titration, respectively, and the structure proposed in Scheme 1.

The mixture of differently substituted homologues was used without separation for the preparation of CDSA/C_{60} complexes.

UV–vis absorption spectra

The UV–vis absorption spectra of CD/C_{60} and CDSA/C_{60} complexes in water solution are presented in Fig. 8. The strong narrow absorptions at 214, 260 and 332 nm for CD/C_{60} and at 214, 258, and 332 nm for CDSA/C_{60}, the spike at 410 and 408 nm for CD/C_{60} and CDAS/C_{60}, and the small broad band centred at about 500 nm confirmed the formation of CD/C_{60} [28] and CDSA/C_{60} complexes. The spectra match those reported by Anderson et al. [29] for CD/C_{60}. It is obvious that the spectra are very close to C_{60} spectrum in *n*-hexane [30], which shows characteristic signals at 211, 256, and 328 nm, a small shoulder at 404 nm and the broad band centred at ca. 550 nm. The resemblance between the spectra of CD/C_{60} or CDAS/C_{60} complexes in water solutions and C_{60} in *n*-hexane clearly shows that there is no chemical modification of C_{60}. The blue shift of the broad band is due to the more polar environment characteristic for water solvent [30]. This broad band is assigned to the HOMO-LUMO transition for C_{60} and consequently the energetic gap is higher for the complex as compared to C_{60} alone in *n*-hexane. Even the resemblance of the spectra of C_{60} in *n*-hexane and the complexes of CD and CDSA in water is obvious; we cannot assume the same absorptivity of C_{60} in *n*-hexane and complexes due to solvatochromic changes and dielectric constant of the solvent [31, 32].

Complexation ratio determined by thermogravimetric analysis

The composition of complexes was roughly calculated for solid complexes from their TGA curves as compared to those corresponding to the non-complexed cyclodextrins and C_{60} precursors after water removal, considering the second step of weight losses (Fig. 9). TGA curves for CD, CDSA and C_{60} are quite different. The decomposition temperatures of CD and CDSA are 325 and 300 °C, respectively, while that for C_{60} is 400 °C. Reading the weight losses corresponding to the known decomposition temperatures of the initial materials, the proportions between the components in the complexes can be determined. For example, for solid CD/C_{60} complex the CD and C_{60} contents were found to be 66% and 34%, respectively, giving a 1:1 ratio between CD and C_{60} (theoretical values for 1:1 complex are 62.42% CD and 34. 68% C_{60}). Similar composition for CD/C_{60} complex was previously reported by Pierre Boulas [24]. In the same manner, the contents of CDSA and C_{60} of about 77% and 23% were determined from the TGA curves and a CDSA/C_{60} ratio of about 1:1 was calculated for the solid CDSA/C_{60} complex (theoretical contents of CDSA and C_{60} in a 1:1 complex are 75.34% and 24.66%, respectively). The composition of the

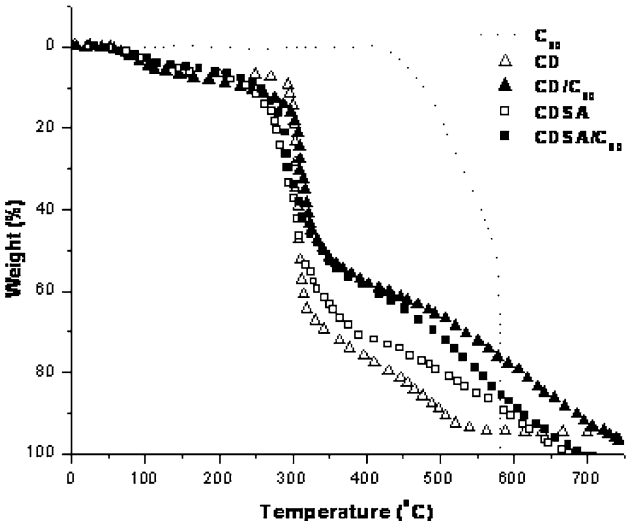


Fig. 9 TGA curves of pure C_{60}, CD and CDSA and their complexes with C_{60}

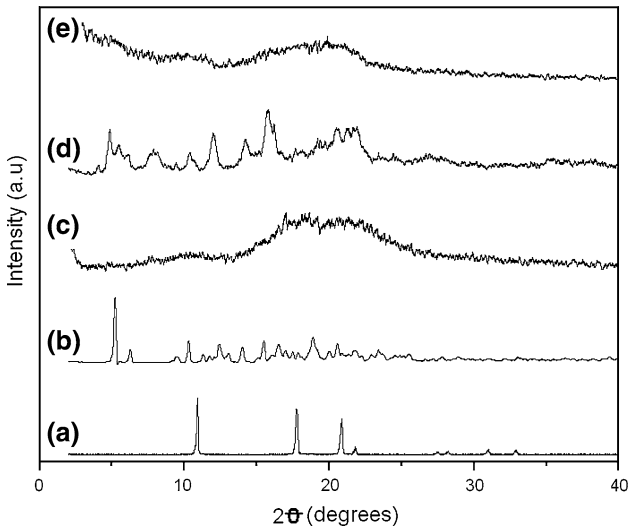


Fig. 10 X-Ray diffraction pattern of CD (**a**), CDSA (**b**), CD/C_{60} complex (**c**) and CDSA/C_{60} complex (**d**)

complexes determined by TGA is in agreement with NMR and potentiometric titration data.

X-ray diffraction pattern

Figure 10 presents a comparison of WAXD data of CD, C_{60}, CDSA, CD/C_{60} and CDSA/C_{60} inclusion complexes at room temperature, at 2θ varying from 1 to 40°. CD and C_{60} samples have highly crystalline structure as indicated by the tiny reflexes of different intensities. Major peaks corresponding to CD are at $2\theta = 12.0, 16.2, 18.6, 21.6, 24.8°$ and the ones of C_{60} are at $2\theta = 10.93, 17.74, 20.85$. They are similar to those reported in literature [33]. The diffraction peaks of CD and CD/C_{60} at low angles (2θ around 7.5°) indicate the formation of the channel type structures [34]. The CD/C_{60} complex shows different diffraction pattern when compared to its CD and C_{60} precursors. On the contrary, CDSA diffraction pattern is a typical one for amorphous products, denoting the complete disturbing of the crystalline structure of CD through its asymmetrical esterification. The same amorphous structure was evidenced for its complex with C_{60}.

Microgel swelling in aqueous solutions of cyclodextrins and of their complexes with C_{60}

It is known that the swelling of polyacrylate hydrogels is sensitive to pH of the solvent. As the aqueous solutions of above described C_{60} complexes with CD and CDSA are intended to be incorporated into such a hydrogel, the dependence of the pH of all studied solutions on their concentrations was first measured. The solutions of the cyclodextrins and those of their complexes were analyzed in concentration range 0–1.0 and 0–0.034 g/dL, respectively. The upper limit of the concentration interval for complex solutions was imposed by their solubility limit in water. As expected, CD and CD/C_{60} solutions exhibit no concentration dependence of pH value, while CDSA and CDSA/C_{60} solutions present a decrease of pH from 6.6 to 3.0 and 4.0, respectively, due to the increase of carboxylic group concentration.

The equilibrium swelling degree (Q) of gel microparticles was studied as a function of solution concentration (Fig. 11). No buffer was used to modify the pH value. The degree of gel swelling at equilibrium in pure water is 310 g/g. The presence of dissolved CD practically does not influence the gel swelling within experimental errors in the studied concentration range (line 1 in Fig. 11a). In CDSA solutions, Q strongly decreases with the increase of

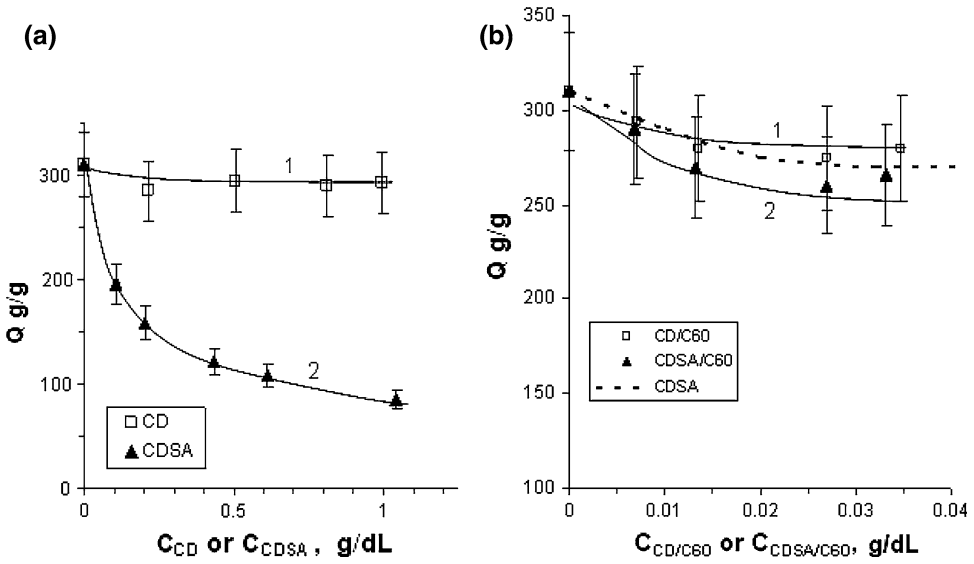


Fig. 11 Variation of the gel swelling degree at equilibrium as a function of (**a**), cyclodextrins and (**b**), complexes concentrations. Lines are given to guide the eye. Dotted line in (**b**) corresponds to the beginning of curve 2 from (**a**)

cyclodextrin concentration due to the increase of solution acidity (Fig. 11a, line 2). A decrease of gel swelling was recorded for CD/C_{60} due to the increase of solution hydrophobicity (line 1 in Fig. 11b) and, by consequence, the decrease of solvent quality, because of the presence of fullerene. Thus, as far as data for CD/C_{60} practically coincide with the ones for CDSA shown by dotted line for comparison (they are the same as in Fig. 11a but taken only at low CDSA concentrations), it seems that the decrease of the solvent quality induced by the presence of C_{60} is rather strong. The complexation of CDSA with fullerene decreases further the gel swelling (Fig. 11b, line 2) because both effects, the hydrophobisation of the solvent and the decrease of pH are acting when CDSA/C_{60} solution is used as gel swelling agent. However, because of the lower solubility of cyclodextrin (modified or not)/C_{60} complexes it is difficult to give quantitative conclusions about the influence of fullerenes on gel swelling degree.

Shear-induced release of cyclodextrins, modified cyclodextrins and their complexes with C_{60} from hydrogels

The release induced by shear of aqueous solutions of CD, CDSA and of their complexes with fullerene C_{60} from microgel particles was studied using rheo-optical counter-rotating set-up [16]. Microgel was swollen to $Q^* = 0.7Q$ in the aqueous solutions of the systems mentioned above. This swelling degree was chosen to ensure complete absorbance of solution by the gel (no free solvent left). The concentration of solutions was the same in all cases, 0.03 g/dL, which is just below the solubility limit for the complexes of C_{60} with CD or CDSA.

A relative volume loss for a microgel particle, ΔV, was monitored as a function of time and shear rate. The diameter of a swollen microgel particle at rest was 0.11–0.16 mm. In our previous study it was shown that the relative volume loss does not depend on the initial size of a particle swollen to the same degree in the same solution [16]. The dependencies of ΔV vs. strain (strain = (shear rate) × time (1/s) × s) for each system were obtained for several particle sizes. The average dependences $\Delta V = f(\text{strain})$ for each solution were then calculated.

The values are presented in Fig. 12. First, up to 70% of solution contained in the gel can be released under shear, whatever is the solution type, with or without C_{60}, and this does not seem to be the limit because the curves are not saturating. Second, the rate of gel volume loss in CDSA solutions and its complex with C_{60} is higher than that in non-modified cyclodextrin and its complex with C_{60}. CD and CD/C_{60} are thermodynamically better solvents for gels as compared with modified cyclodextrin and its complex, because of CDSA acidity. Thus CDSA

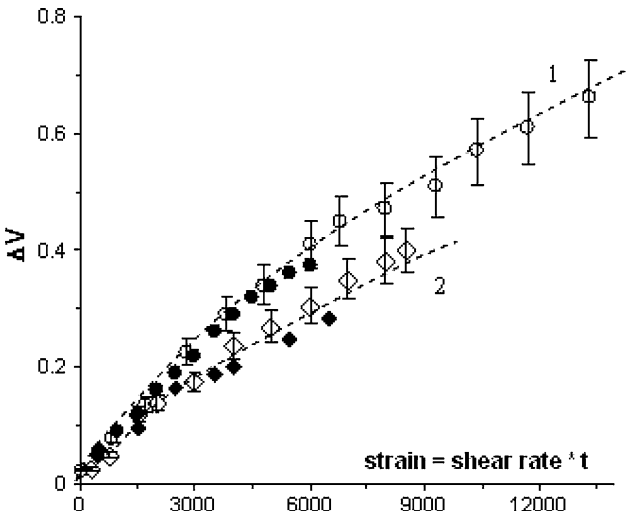


Fig. 12 Relative volume loss of a microgel swollen in aqueous solutions of CDSA (line 1, open points); CDSA/C_{60} (line 1, solid points); CD (line 2, open points); CD/C_{60} (line 2, solid points) as a function of strain. Lines are given to guide the eye

and CDSA/C_{60} are easier to "extract" from the gel. No significant influence of C_{60} on the release from microgel was noticed. This can be probably explained by very dilute solution concentration: a slight decrease in solvent thermodynamic quality due to the presence of hydrophobic C_{60} is masked by rheo-optical experimental errors that are around 10%.

The microgels swollen in solutions of fullerene inclusion complexes with CD and CDSA can be used as appropriate carriers for fullerene controlled delivery. The release of fullerene-containing inclusion complexes can be varied in a wide range of gel volume loss: from a small volume loss to almost complete deswelling by means of strain variations, i.e. time or/and shear rate change.

Determination of the C_{60} CDSA complex composition by titration of carboxylic groups in CDSA and CDSA/C_{60}complex

As observable in Fig. 13 a and b the titration curves of carboxylic groups in CDSA and CDSA/C_{60} complex are similar to those characteristic for a week monoprotic acid, and the equivalence point can be estimated from the inflection point in the titration curve.

The equivalence point determined from Fig. 13 a represents the degree of substitution/mol CD. Applying the Eq. 1 we obtained an average molar content equal to 9.6 carboxylic groups/mol CD.

The equivalence point determined from Fig. 13 b and that determined from Fig. 13 a allows the determination of the number of CD with carboxylic groups/mol of C_{60}, the molar ratio CDAS/C_{60} is 1:1.

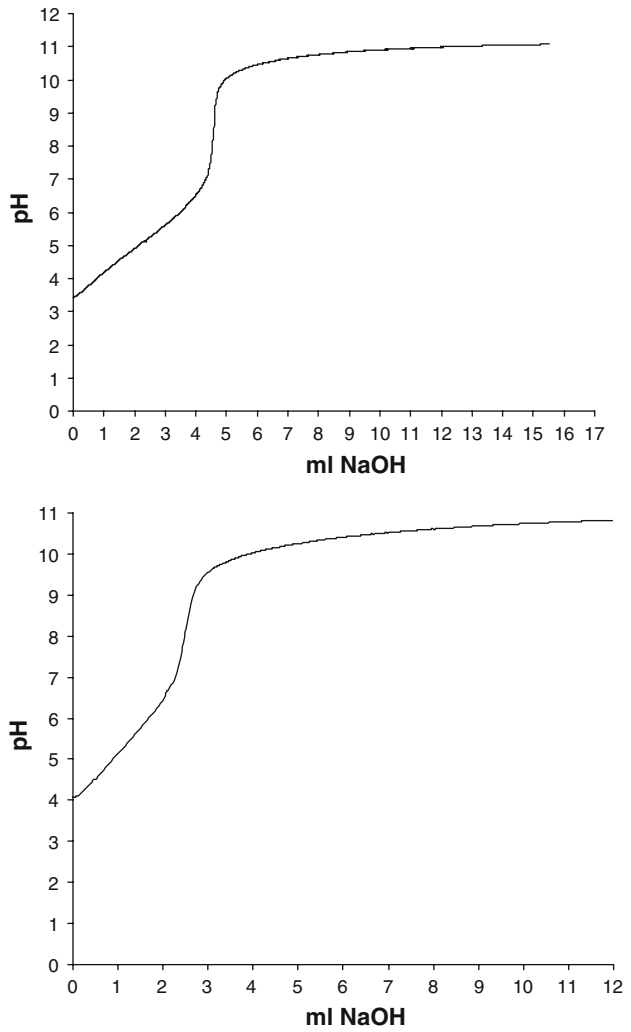


Fig. 13 The titration curve for CDSA (**a**) and CDSA/C_{60} complex with NaOH solution

Conclusions

The preparation of succinic acid derivative of γ-cyclodextrin with a substitution degree of about 9.5 and the complexation of C_{60} with γ-cyclodextrin and carboxyl-modified γ-cyclodextrin performed in water–toluene solvents mixture is described in the paper. Potentiometric titration, NMR and TGA demonstrate the formation of 1:1 = CD:C_{60} and 1:1 = CDSA:C_{60} inclusion complexes. The asymmetric modification of the cyclodextrin induces the decrease of its crystallinity, as well as of the crystallinity of its C_{60} complex, as compared to the non modified corresponding compounds. Aqueous solutions of native cyclodextrin, carboxyl-modified cyclodextrin and their inclusion complexes with C_{60} were used as solvents to swell cross-linked poly(sodium acrylate-acrylic acid) microgel. A strong decrease of microgel swelling with increasing concentration of carboxyl modified cyclodextrin was noticed due to the concomitant decrease of the solution pH. The shear-induced release of fullerene-containing solutions from the microgel was demonstrated using rheo-optics. More than 70% of gel initial volume can be released in about 25 min after shearing at 10 s^{-1}.

Acknowledgements Part of this work was supported by Egide ECO-NET program (France), project N 12556 WC. The authors are also grateful to the Russian Foundation for Basic Research (project No 07-03-91681-PA_a) and the Romanian Academy (project No 03/2008) for a partial financial support.

References

1. Jensen, A.W., Wilson, S.R., Schuster, D.I.: Biological applications of fullerenes. Bioorg. Med. Chem. **4**(6), 767–779 (1996). doi:10.1016/0968-0896(96)00081-8
2. Ungurenasu, C., Pinteala, M., Simionescu, B.C.: Synthesis and characterization of nitrogen-bridged [C_{60}] fullerene/3′-deoxythimidine conjugates. Synthesis **3**, 361–363 (2005). doi:10.1055/s-2005-861794
3. Schinazi, R.F., Sijbesma, R., Srdanov, G., Hill, C.L., Wudl, F.: Synthesis and virucidal activity of a water-soluble, configurationally stable, derivatized C_{60} fullerene. Antimicrob. Agents Chemother. **37**(8), 1707–1710 (1993)
4. Williams, R.M., Zwier, J.M., Verhoeven, J.W.: Interactions of fullerenes and calixarenes in the solid state studied with ^{13}C CP-MAS NMR. J. Am. Chem. Soc. **116**(15), 6965–6966 (1994). doi:10.1021/ja00094a073
5. Haino, T., Yanase, M., Fukazawa, Y.: Crystalline supramolecular complexes of C_{60} with calix[5]arenes. Tetrahedron Lett. **38**(21), 3739–3742 (1997). doi:10.1016/S0040-4039(97)00741-7
6. Barbour, L.J., Orr, G.W., Atwood, J.L.: Supramolecular intercalation of C_{60} into a calixarene bilayer—a well-ordered solid-state structure dominated by van der Waals contacts. Chem. Commun. (Camb.) **15**, 1439–1440 (1997). doi:10.1039/a702075i
7. Takekuma, S., Takekuma, H., Matsumoto, T., Yoshida, Z.: A highly efficient generation of γ-cyclodextrin-bicapped C_{60}^{-1} in aqueous solution. Tetrahedron Lett. **41**(25), 4909–4912 (2000). doi:10.1016/S0040-4039(00)00717-6
8. Ala-Kleme, T., Maki, R., Haapakka, K.: Anomalous luminescence of a bicapped [60]fullerene-γ-cyclodextrin complex at an oxide-covered aluminium cathode. Phys Chem Chem Phys **4**(13), 3055–3060 (2002). doi:10.1039/b108679k
9. Ala-Kleme, T., Maki, R., Laaksonen, P., Haapakka, K.: Blue and red photoluminescence of bicapped [60]fullerene-γ-cyclodextrin complex in aqueous solutions. Anal. Chim. Acta **472**(1–2), 83–87 (2002). doi:10.1016/S0003-2670(02)00941-8
10. Ungurenasu, C., Airinei, A.: Highly stable C_{60}/Poly(vinylpyrrolidone) charge-transfer complexes afford new predictions for biological applications of underivatized fullerenes. J. Med. Chem. **43**(16), 3186–3188 (2000). doi:10.1021/jm991175q
11. Vinogradova, L.V., Melenevskaya, E.Y., Khachaturov, A.S., Kever, E.E., Litvinova, L.S., Novokreshchenova, A.V., Sushko, M.A., Klenin, S.I., Zgonnik, V.N.: Water-soluble complexes of C_{60} fullerene with poly(N-vinylpyrrolidone). Polym. Sci. A **40**(11), 1152–1160 (1998)
12. Murthy, C.N., Geckeler, K.E.: The water-soluble β-cyclodextrin–[60]fullerene complex. Chem. Commun. (Camb.) **13**, 1194–1195 (2001). doi:10.1039/b102142g
13. Belgorodsky, B., Fadeev, L., Kolsenik, J., Gozin, M.: Formation of a soluble stable complex between pristine C_{60}-fullerene and a

native blood protein. ChemBioChem **7**(11), 1783–1789 (2006). doi:10.1002/cbic.200600237
14. Eddaoudi, H., Deratani, A., Tingri, S., Sinan, F., Seta, P.: Fullerene membrane transport in PVA films. Polym. Int. **52**(8), 1390–1395 (2003). doi:10.1002/pi.1240
15. Bodor, N., Huang, M.J., Watts, J.D.: Theoretical am1 studies of inclusion complexes of α- and β-cyclodextrins with methylated benzoic acids and phenol, and γ-cyclodextrin with buckminsterfullerene. J. Incl. Phenom. Macrocycl. Chem. **25**(1–3), 97–102 (2005). doi:10.1007/BF01041545
16. Liu, Y., Wang, H., Liang, P., Zhang, Y.H.: Water-soluble supramolecular fullerene assembly mediated by metallobridged beta-cyclodextrins. Angew. Chem. Int. Ed. **43**(20), 2690–2694 (2004). doi:10.1002/anie.200352973
17. Ying, Y., Zhiqiang, S., Yongshan, M., Mei, X., Jiechao, G.: Highly water-soluble [60]Fullerene-ethylenediamino- + ¦-cyclodextrin inclusion complex: the synthesis and self-assembly with poly (Acrylic Acid). Supramol. Chem. **20**(3), 295–299 (2008). doi:10.1080/10610270701242372
18. Boger, J., Corcoran, R.J., Lehn, J.M.: Cyclodextrin chemistry selective modification of all primary hydroxyl groups of α- and β-cyclodextrins. Helv. Chim. Acta **61**, 2190–2218 (1978). doi:10.1002/hlca.19780610622
19. Takeo, K., Mitouh, H., Uemura, K.: Selective modification of cyclomalto-oligosccharides *via* *tert*-butyldimethylsilylation. Carbohydr. Res. **187**(2), 203–221 (1989). doi:10.1016/0008-6215(89)80004-7
20. Zeo, U., Tarabukina, E., Budtova, T.: Kinetics of shear-induced gel deswelling/solvent release. J. Control Release **108**(1), 73–83 (2005). doi:10.1016/j.jconrel.2005.07.012
21. Gonzalez, C.J., Rossi, R.H.: Synthesis and complexation properties of an amphiphilic cyclodextrin. Arkivoc **XII**, 87–99 (2001)
22. Zanina, A., Budtova, T.: Hydrogel under shear: a rheo-optical study of the particle deformation and solvent release. Macromolecules **35**(5), 1973–1975 (2002). doi:10.1021/ma011425o
23. Vervoort, S., Budtova, T.: Shear-induced solvent release from a swollen microgel in the vorticity direction. Colloid Surf. A Physicochem. Eng. Asp. **262**(1–3), 132–138 (2005). doi:10.1016/j.colsurfa.2005.04.013
24. Boulas, P., Kutner, W., Jones, M.T., Kadish, K.M.: Bucky basket ball stabilization of electrogenerated $C60^{\bullet-}$ radical monoanion in water by means of cyclodextrin inclusion chemistry. J. Phys. Chem. **98**(4), 1282–1287 (1994). doi:10.1021/j100055a039
25. Schneider, H.J., Hacket, F., Rudiger, V.: NMR studies of cyclodextrins and cyclodextrin complexes. Chem. Rev. **98**(5), 1755–1785 (1998). doi:10.1021/cr970019t
26. Cucinotta, V., Giuffrida, A., Maccarrone, G., Messina, M., Puglisi, A., Torrisi, A., Vecchio, G.: The 6-derivative of β-cyclodextrin with succinic acid: a new chiral selector for CD-EKC. J. Pharm. Biomed. Anal. **37**(5), 1009–1014 (2005). doi:10.1016/j.jpba.2004.08.023
27. Anderson, T., Westman, G., Wennerstrom, O., Sundahl, M.: NMR and UV-VIS investigation of water-soluble fullerene–60-γ-cyclodextrin complex. J. Chem. Soc., Perkin Trans. **2**(5), 1097–1101 (1994) doi:10.1039/p29940001097
28. Priyadarsini, K.I., Mohan, H., Tyagi, A.K., Mittal, J.P.: Inclusion complex of γ-cyclodextrin-C_{60} formation, characterization, and photophysical properties in aqueous solutions. J. Phys. Chem. **98**(17), 4756–4759 (1994). doi:10.1021/j100068a044
29. Anderson, T., Nilsson, K., Sundahl, M., Westman, G., Wennerstrom, O.: C_{60} embedded in γ-cyclodextrin: a water-soluble fullerene. J. Chem. Soc. Chem. Commun. 8, 604–606 (1992)
30. Ajie, H., Alvarez, M.M., Anz, S.J., Beck, R.D., Diederich, F., Fostiropoulos, K., Huffman, Wolfgang, D.R., Kratschmer, W., Rubin, Y., Schriver, K.E., Sensharma, D., Whetten, R.L.: Characterization of the soluble all-carbon molecules C_{60} and C_{70}. J. Phys. Chem. **94**(24), 8630–8633 (1990) doi:10.1021/j100387a004
31. Sun, Y.P., Ma, B., Bunker, C.E., Liu, B.: All-carbon polymers (Polyfullerenes) from photochemical reactions of fullerene clusters in room-temperature solvent mixtures. J. Am. Chem. Soc. **117**(51), 12705–12711 (2008). doi:10.1021/ja00156a007
32. Mrzel, A., Mertelj, A., Omerzu, A., Cyopic, M., Mihailovic, D.: Investigation of encapsulation and solvatochromism of fullerenes in binary solvent mixtures. J. Phys. Chem. B **103**(51), 11256–11260 (2008)
33. Tseng, W.Y., Chen, Y.H., Khairullin, I.I., Cheng, S., Hwang, L.P.: NMR study of solid C_{60}(γ-cyclodextrin)$_2$. Solid State Nucl. Mag. **8**(4), 219–229 (1997). doi:10.1016/S0926-2040(97)00013-1
34. Marangoci, N., Fifere, A., Farcas, A., Perichaud, A., Harabagiu, V., Pinteala, M., Simionescu, B.C.: Synthesis and characterization of polyrotaxanes based on cyclodextrins and viologen modified polydimethylsiloxanes. High Perform. Polym. Online-First. Published on 11 December 2007 as doi:10.1177/0954008307082152