Supramolecular complexes of spin-labelled cyclodextrins

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Received 30th May 2006, Accepted 7th July 2006
First published as an Advance Article on the web 7th August 2006
DOI: 10.1039/b607676a

EPR spectra of cyclodextrins labelled with TEMPO derivatives (SL-CDs) are sensitive to complexation with large guest molecules. We used SL-CDs to explore the behaviour of concentrated PEG/PPG solutions. The relationship between rotational correlation times and solvent viscosity showed significant deviations from the Debye–Stokes–Einstein equation, probably due to self-aggregation of alkylene glycols in concentrated solutions. The data fit the fractional Debye–Stokes–Einstein equation well. We have also studied complexation of SL-CDs with adamantane-functionalised DAB dendrimers. The strength of binding increases with dendrimer generation; formation of supramolecular aggregates at high concentrations was observed with the generation 3 dendrimer.

Introduction

Cyclodextrins (CDs) are rigid molecules with a hydrophobic cavity and hydrophilic exterior which determine their ability to bind various low molecular weight compounds and parts of larger molecules, both in solution and in the solid state.[1,2] Host properties of CDs are exploited in the practical applications in food and pharmaceutical industries, in modelling of enzymatic processes, chromatography and capillary electrophoresis.[3] Recent examples of supramolecular chemistry of CDs include construction of molecular machines based on rotaxanes,[4] polyrotaxanes,[5–7] catenanes,[8] and design of molecular printboards prepared by self assembly of CD derivatives on different surfaces.[9]

Inclusion complexes and molecular assemblies of CDs can be studied by a variety of analytical methods which provide information on kinetics and thermodynamics of association and on the structure of supramolecular assemblies. We are interested in using EPR spectroscopy to probe the structure and dynamics of supramolecular assemblies of CDs. EPR is particularly suitable for studying supramolecular interactions as this technique is sensitive to local structure in the vicinity of spin labels and molecular dynamics on the nanosecond timescale. EPR can of course only be used if either the host or the guest molecules are paramagnetic.

There have been many reports of the inclusion complexes of paramagnetic species with cyclodextrins studied by EPR spectroscopy. The interaction of sterically protected, stable free radicals (*e.g.*, TEMPO derivatives) with CDs leads to a smaller nitrogen hyperfine splitting a_N, which indicates that the radical is in a less polar environment than pure water (*e.g.*, in the CD cavity). Unfortunately, the differences are small, and EPR spectra cannot provide a clear distinction between free and complexed TEMPO radicals.[10] Complexation of other free radicals, however, can often be observed. For instance, Kotake and Janzen were able to characterise the interaction between diphenylmethyl *tert*-butyl nitroxide with CDs.[11–13] Formation of inclusion complexes of CDs with nitroarene radical anions[14] and *N*-alkylphenothiazine radical cations has also been reported.[15] Tordo *et al.* studied trapping of oxygen-centred radicals with nitrones in the presence of CDs. Formation of inclusion complexes in this case led to an increase of their half-life.[16–18] Lucarini *et al.* also explored inclusion complexes formed between organic radicals and CDs.[19,20]

Attaching a paramagnetic moiety to cyclodextrin makes it possible to expand the scope of EPR studies to complexes of CDs with unlabelled molecules. We have previously reported the synthesis of three spin-labelled cyclodextrins (SL-CDs, Fig. 1). Unfortunately, we found that complexation of these probes with small molecules did not lead to appreciable changes of the EPR spectra. However, EPR can detect incorporation of SL-CDs in large supramolecular assemblies.[21]

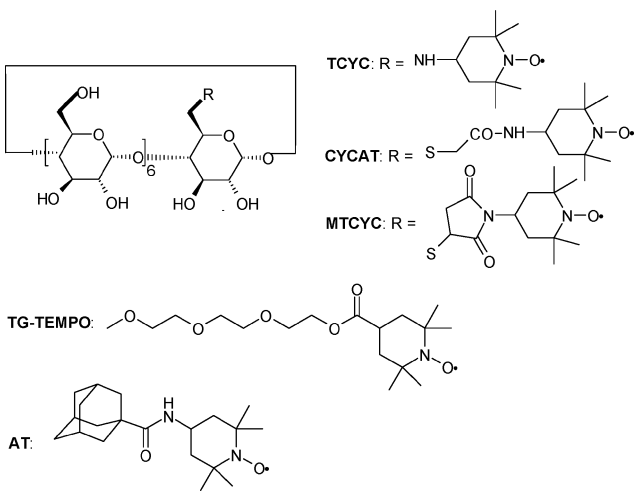

Fig. 1 Structure of spin labels.

In this paper, we examined the interaction of SL-CDs with two types of "large" molecules: polyethylene and polypropylene glycols in concentrated aqueous solutions, and adamantane-functionalised dendrimers.

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Results and discussion

Interaction of SL-CDs with PEG 600 and PPG 425

Aqueous solutions of polyethylene glycols (PEGs) have attracted much interest. They were studied using ultrasonic techniques, photon correlation spectroscopy, NMR, dynamic and static light scattering, viscosity measurements.[22–26] Importantly, some reports conclude that aqueous solutions of PEGs are not homogeneous but contain polymer aggregate (or clusters) and free polymer which coexist in a thermodynamic equilibrium depending on the solvent, temperature, concentration.[22,23]

Polyalkylene glycols often form crystalline inclusion complexes with CDs. For instance, polypropylene glycols (PPGs) form such complexes with β-CD and γ-CD, PEG with α-CD.[2,28,29] However, some of these complexes (including β-CD with PEG) are soluble in water. We have studied the interaction of PEG and PPG with SL-CDs and a model spin probe TG-TEMPO (Fig. 1) in concentrated aqueous solutions.

EPR spectra of these solutions showed the progressive immobilisation of the spin probe with increased PEG concentration (Fig. 2). EPR parameters for PEG 600 and PPG 425 solutions are shown in Tables 1 and 2, respectively. The spectral parameters for all spectra were calculated using Kivelson theory[27] except MTCYC. MTCYC in 40 and 50% PEG/PPG solutions showed spectra typical of slow motion. Rotational correlation times (τ) for all MTCYC solutions were therefore calculated from the diffusion rates obtained by spectra simulation with the NLSL programme.[30] Simulation were performed using literature values for the A and g tensors (except A_{zz} which was optimised during the fit).[31] Good fits were obtained by assuming a simple model of isotropic Brownian diffusion.

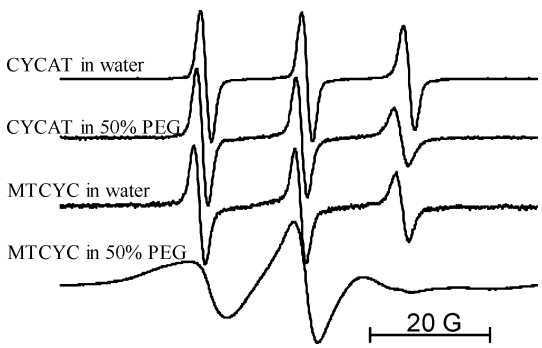

Fig. 2 EPR Spectra of CYCAT and MTCYC in water and PEG 600 solutions.

We assumed that most SL-CD is complexed with PEG molecules. Binding constants for similar complexes between native β-CD and PEG are *ca.* 10^5 M^{-1}.[32] The concentration of SL-CD was 1 mM, so PEG molecules were in large excess.

The a_N values decrease with increased concentration of PEG 600 which is consistent with the reduced hydrophilicity of the concentrated PEG solutions (Table 1). The same behaviour is observed in the case of PPG 425 (Table 2). Interestingly, the mobility of the three SL-CDs is significantly different. MTCYC has the longest rotational correlation time (τ). In 40% and 50% PEG/PPG solutions, MTCYC has spectra typical of slow motion (Fig. 2), while all other spectra show fast motion. This is because the linker between the nitroxide and the CD in this molecule is quite rigid, and hence the spin probe reports on the movement of the whole molecule (*e.g.*, MTCYC–PEG complex). On the other hand, in CYCAT and TCYC the linker is much more flexible, and therefore the τ values are strongly affected by the local motion. For example, the τ values for solutions of CYCAT in PEG vary in a relatively narrow range (from 3.11 to 8.30) compared to much more rigid MTCYC.

The τ values can be correlated with the solution viscosity η and hydrodynamic radius of the probe R_h according to the Debye–Stokes–Einstein relationship [eqn (1)]:[33]

$$\tau = \frac{4\pi\eta R_h^3}{3kT} \quad (1)$$

However, application of this equation to the data in Tables 1, 2 (and literature data on viscosity and density for PEG[26] and PPG[34]) showed that the relationship between the rotational correlation time and viscosity is not linear. The deviation from eqn (1) is commonly observed for mixed solvents, and is often attributed to non-Brownian motion, specific solvent–solute interactions, change in the hydrodynamic boundary conditions (*e.g.*, if solvent mixture is inhomogeneous).[35] The data analysis in these cases is usually carried out using fractional Debye–Stokes–Einstein relationship [eqn (2)]:[36]

$$\frac{\tau}{\tau_s} = \left(\frac{\eta}{\eta_s}\right)^q \quad (2)$$

Here, the relative values of rotational correlation time (*e.g.*, relative to a pure solvent) are correlated with relative viscosity. The scaling factor q depends on both the solvent and solute molecules.

Table 1 Rotational correlation times τ and hyperfine splittings a_N of SL-CDs in concentrated PEG 600 aqueous solutions

	TG-TEMPO		TCYC		CYCAT		MTCYC[a]
PEG 600%	a_N/G	$\tau \times 10^{10}$/s	a_N/G	$\tau \times 10^{10}$/s	a_N/G	$\tau \times 10^{10}$/s	$1/(6D) \times 10^{10}$/s
0	17.05	0.54	16.77	4.55	16.86	3.11	4.19
5	17.03	0.58	16.73	5.58	16.79	3.75	4.81
10	17.01	0.69	16.69	6.80	16.84	4.00	5.70
15	16.98	0.78	16.68	7.98	16.73	4.31	8.01
20	16.94	0.86	16.61	8.99	16.81	4.47	9.29
30	16.89	1.17	16.57	14.63	16.79	5.32	13.80
40	16.80	1.60	16.52	21.34	16.70	6.29	24.20
50	16.66	2.42	16.33	31.9	16.60	8.30	34.98

[a] Calculated from the isotropic diffusion rate D. The τ value for MTCYC in water calculated according to ref. 27 is 7.74.

Table 2 Rotational correlation times τ and hyperfine splittings a_N of SL-CDs in concentrated PPG 425 aqueous solutions

	TG-TEMPO		TCYC		CYCAT		MTCYC[a]
PPG 425%	a_N/G	$\tau \times 10^{10}$/s	a_N/G	$\tau \times 10^{10}$/s	a_N/G	$\tau \times 10^{10}$/s	$1/(6D) \times 10^{10}$/s
5	17.01	0.78	16.85	5.43	17.01	3.10	4.86
10	16.99	0.81	16.84	6.38	16.99	3.26	5.10
15	16.89	1.07	16.85	6.75	16.99	3.80	7.03
20	16.86	1.24	16.83	7.08	16.98	4.52	7.36
30	16.75	1.88	16.81	8.42	16.89	4.93	14.85
40	16.60	2.71	16.73	9.16	16.77	7.00	25.64
50	16.40	4.15	16.60	12.98	16.65	9.39	32.50

[a] Calculated from the isotropic diffusion rate D. The τ value for MTCYC in water calculated according to ref. 27 is 7.74.

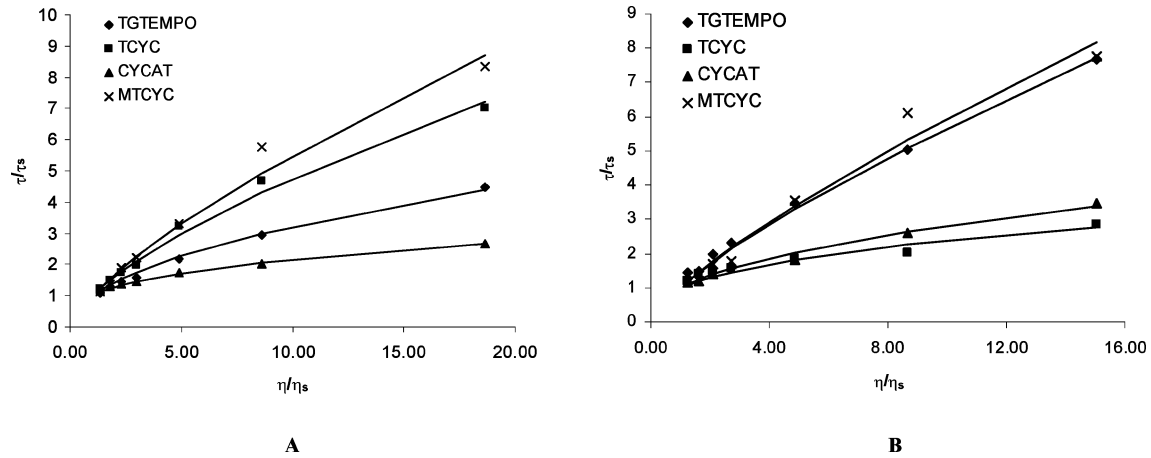

Fig. 3 Fractional Debye–Stokes–Einstein relationship for spin-labelled cyclodextrins in PEG 600 (**A**) and PPG 425 (**B**) solutions.

Eqn (2) showed good fit to our experimental data (Fig. 3). Interestingly, the deviation from eqn (2) is significantly bigger for MTCYC and TCYC as compared to CYCAT and TG-TEMPO. We believe that this deviation is related to the flexibility of the linker connecting the spin probe to the rest of the molecular assembly. In the case of TG-TEMPO and CYCAT, the nitroxide dynamics report on the local environment, "microviscosity", which is considered by the Debye–Stokes–Einstein equation. In MTCYC and TCYC the linker is much more rigid, the nitroxide is strongly attached to the cyclodextrin and hence to the molecule of cosolvent (PEG or PPG). The tumbling of these species therefore can no longer be approximated by Brownian motion of a spherical probe.

The values of the scaling parameter q were 0.51 (0.75), 0.68 (0.38), 0.33 (0.45), and 0.74 (0.77) for TG-TEMPO, TCYC, CYCAT, and MTCYC in PEG (PPG) solutions, respectively. We believe that such big variation of the scaling factor with the structure of the spin label is best explained by the non-homogeneous nature of concentrated aqueous PEG solutions. Aggregation of PEG chains would affect SL-CDs differently, due to the different chain length and flexibility of the linker. Hence one could expect different scaling factors for these molecules. Aggregation of PEG in aqueous solutions has also been proposed in the literature.[23–26]

Our data are thus best rationalised as follows. We have earlier proposed that in aqueous solution SL-CDs form self-inclusion complexes. In PEG/PPG solutions, this self-inclusion equilibrium is perturbed by host–guest interaction with the polymer molecules. This process is further complicated by the aggregation of PEG/PPG chains, so that the environment around the spin probes is less viscous compared to the average viscosity in the bulk solution.

Interaction of SL-CDs with adamantane-functionalised dendrimers

In our earlier communication,[21] we found that EPR parameters of SL-CDs do not change significantly upon interaction with small guest molecules. For instance, complexation between SL-CDs (10^{-3} M) with adamantanamine (1 mM–1 M) leads to a very small increase of the a_N value (0.05–0.1 G) and a small decrease in τ (15–20%). These changes can be explained if we consider that complexation of SL-CD with a guest is in competition with the self-inclusion process. Upon host–guest complexation, the paramagnetic moiety is pushed out of the cyclodextrin cavity into a more polar surrounding. Unfortunately, the spectral changes were too small for a reliable analysis of the complexation.

In order to probe the complexation of the adamantane moiety with CDs, we prepared spin labelled adamantane (AT, Fig. 1) and explored its interactions with unlabelled CDs. Table 3 shows the EPR parameters for the AT (saturated solution, *ca.* 0.1 mM) at different concentrations of β-CD. One can see that the τ value increases abruptly upon addition of β-CD but then continues to increase further with the increased concentration of β-CD. At the same time, the a_N value is not influenced significantly until 5×10^{-3} M of β-CD, which implies that the polarity of the paramagnetic moiety environment does not change. We believe

Table 3 EPR parameters of AT at different concentration of β-CD

	[β-CD]					
	0	10^{-4}	5×10^{-4}	10^{-3}	5×10^{-3}	10^{-2}
a_N/G	17.03	17.02	17.00	17.00	17.03	16.93
$\tau \times 10^{10}$/s	0.83	2.50	2.73	2.78	2.98	3.46

that these observations can be explained by sequential complexation of the adamantane and TEMPO units of the AT molecule. The binding constants of adamantanes with β-CD are usually around 10^4–10^5 M^{-1},[37] while for the TEMPO derivatives these values are significantly lower (10^2–10^3 M^{-1}).[10–14] Hence at lower concentrations of β-CD, the complexation of the adamantane moiety is most likely. This leads to the reduction of the spin probe mobility (τ value, Table 3), but does not change the polarity in the vicinity of the TEMPO unit. At higher concentrations of β-CD, formation of the 1 : 2 complex is likely (*e.g.*, with both adamantane and TEMPO units complexed by the CD cavities), which leads to reduction of the polarity (*e.g.*, lower a_N value). Although the complexation of adamantane with the CD cavity has certainly taken place, the changes in the EPR parameters are again too small for quantitative analysis.

In order to increase the sensitivity of the EPR parameters to host–guest complexation of SL-CDs, we prepared a series of adamantane-functionalised poly(propylene imine) dendrimers. The large size of the dendrimer molecules should lead to a significant increase of the τ values of SL-CDs upon complexation. The dendrimers were functionalised with several adamantane groups following a literature procedure.[38,39] The average number of adamantane moieties per dendrimer was calculated from the relative intensity of the NMR signals of CH_2NHCO group. The functionalised 1st, 2nd and 3rd generation dendrimers were labelled ADAB8, ADAB16 and ADAB64; these molecules had 2.8, 4.0 and 4.4 adamantane groups per dendrimer, respectively.

We recorded EPR spectra for TCYC at pH 7 in the presence of different concentrations of each functionalised dendrimer. An increase in the τ values was observed for all dendrimers; however the changes were most pronounced for ADAB16 (Fig. 4). The relatively similar changes in τ for ADAB8 and ADAB64 are probably due to the significant flexibility of the dendrimers. The immobilisation of the TCYC molecule upon complexation (as reported by the τ values) depends primarily on the mobility of the dendrimer branch (*e.g.*, local motion), and not on the overall size of the dendrimer molecule (*e.g.*, the spin label does not feel the tumbling of the dendrimer as a whole). Similar phenomena were reported for other related systems.[40,41]

The values of τ can be used to calculate binding constants for the complexation of adamantane functionalised dendrimers with TCYC. We found that for the mixture of two species (*e.g.*, bound and unbound dendrimer), τ values are linearly proportional to the composition of the mixture. This makes it possible to estimate binding constants by regression analysis assuming formation of a simple 1 : 1 complex between the adamantane moiety and the cyclodextrin.

Fig. 4 shows the variation of τ values for TCYC in the presence of functionalised dendrimers. The data for ADAB8 and ADAB64 dendrimers can be fitted well with the simple binding model. However, complexation of ADAB16 with TCYC showed much higher τ values, particularly at high concentrations of TCYC. This concentration dependence of τ can only be explained by formation of aggregates in solution. We assume that this aggregation follows the complexation between CD cavities and adamantane moieties (Fig. 5). It is not clear why aggregation is only observed with ADAB16, and not with the higher or lower generation dendrimers.

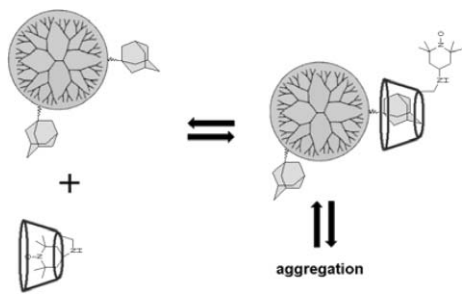

Fig. 5 Aggregation in the mixture of TCYC–ADAB16.

The binding constants calculated from the data in Fig. 4 were 160, 700 and 950 M^{-1} for ADAB8, ADAB16 (calculated for the lowest concentration of TCYC where aggregation is minimal) and ADAB64, respectively. It is interesting to see that binding strength increases with dendrimer generation. However, all association

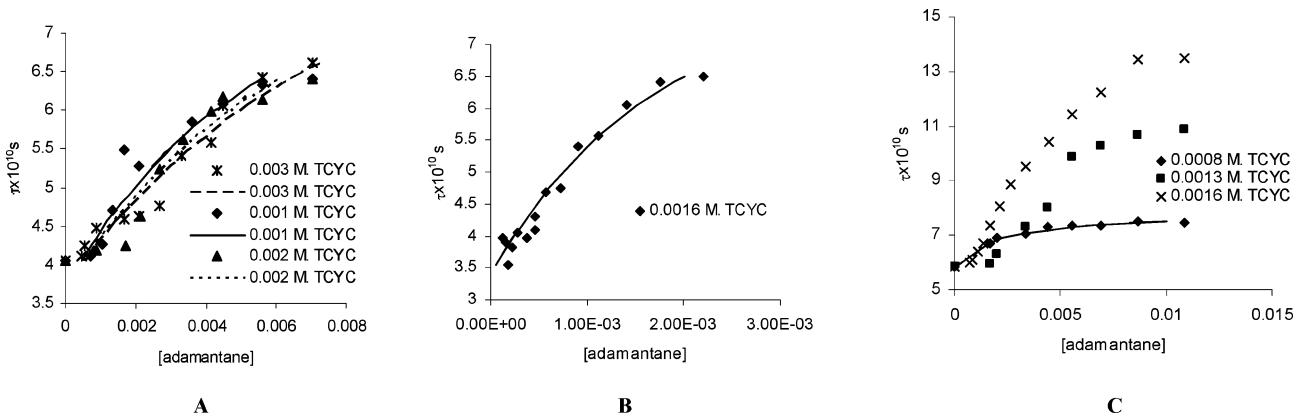

Fig. 4 Experimental τ values (data points) and best fits (lines) for mixtures of TCYC with adamantane-functionalised DAB dendrimers ADAB8 (**A**), ADAB64 (**B**) and ADAB16 (**C**).

constants are much smaller than typical values for adamantane–β-CD complexation. We believe this is due to the self-inclusion of TCYC.

Experimental

Methods

^{1}H-NMR spectra were recorded on a JEOL EX270 NMR spectrometer. ESI mass spectra were measured on a VG Autospec mass spectrometer. EPR spectra were recorded on a JEOL JESRE1X spectrometer with a 100 kHz modulation frequency, a microwave power of 1 mW and a modulation amplitude of 0.1 mT.

Materials

Solvents and chemicals were purchased from Aldrich (DAB-Am-8, DAB-Am-16 and DAB-Am-64, PEG 600, PPG 425, *N*-hydroxysuccinimide, adamantane carbonyl chloride), Fluka (DCC), Avocado (adamantane carboxylic acid) and used as received.

Succinimidoyl adamantane carboxylate was synthesised from adamantane carboxylic acid and *N*-hydroxysuccinimide using a standard protocol for DCC coupling.[39]

Fully adamantane-functionalised DAB dendrimers were synthesised according to a literature procedure.[38] Partially adamantane-functionalised DAB dendrimers were obtained by mixing solutions of DAB-dendr-$(NH_2)_n$ (1 mmol) in CH_2Cl_2 (10 ml) with the appropriate amount of succinimidoyl adamantane carboxylate. The reaction was stirred for 24 h and subsequently an equal volume of a NaOH solution (1 M) was added. After one day, the organic phase was extracted and the solvent was evaporated in vacuum.

Adamantane-TEMPO (AT) was prepared by adding adamantanecarbonyl chloride (0.198 g, 1 mmol) to a solution of 4-amino-TEMPO (0.171 g, 1 mmol) in DCM in the presence of few drops of pyridine. After stirring for 1 day, the reaction mixture was washed with aq. HCl to extract the unreacted 4-amino-TEMPO. The organic phase was separated and the solvent was evaporated in vacuum to yield the crude product (yield 80%), which was purified by column chromatography. ESI-MS, *m*/*z*: 332 (100%, AT − H^+, negative ions) or 356 (100%, AT + Na^+, positive ions). EPR, a_N: 17.08 G (H_2O).

TG-TEMPO was prepared by mixing carboxy-TEMPO (220 mg, 1.1 mmol) with a solution of triethylene glycol monomethyl ether (0.164 g, 1 mmol) in DCM in the presence of DCC (251 mg, 1.2 mmol) and DMAP (1 mmol). After stirring for one day at room temperature, the reaction mixture was washed successively with 0.1 M HCl and aq. $NaHCO_3$ solutions. The organic layer was dried (Na_2SO_4). The solution was concentrated and TG-TEMPO was purified by column chromatography (10% ethyl acetate–DCM) to yield 0.170 mg (46%). ESI-MS, *m*/*z*: 369 (100%, TG-TEMPO + Na^+).

Conclusions

Spin labelled cyclodextrins make it possible to use EPR spectroscopy to monitor various supramolecular interactions, particularly with large guest molecules. This system may help address a number of important issues, including formation of mesoporous materials using cyclodextrins or PEG–cyclodextrin complexes as templates, interactions of cyclodextrin with surfactants (especially at higher concentrations) and biological molecules.

Acknowledgements

Support of this work was provided by the Royal Society (UK), NATO, CNCSIS and MEC-Romania.

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