CHAPTER 12

Nano-Chemistry: Stabilised nano-arrays of fullerene C_{60} and C_{70} in water using p-sulfonatocalix[4]arene

Stable aqueous colloidal suspensions of fullerene C_{60} and C_{70} in the range 40 to 130 nm are readily accessible by mechanical grinding of a mixture of a fullerene and p-sulfonatocalix[4]arene, also in the presence of a sulfonato-imidazloum, followed by ultrasonication in water. The yellow and brown solutions for C_{60} and C_{70} respectively, are stable in the presence of toluene, and were characterized using UV-visible, fluorescence, FTIR and Raman spectroscopy, with the nano-particles characterised using dynamic light scattering, thermogravimetric analysis and transmission electron microscopy.

12.1 Introduction

Fullerenes have gained much attention because of their potential biological activity, such as HIV-1 protease inhibition, photodynamic tumor necrosis, and as artificial vectors for gene transfection [315-317]. In this context a challenge is the preparation of stable aqueous solutions of pristine fullerenes, either as discrete host-guest complexes or as nano-particles of the fullerenes. Fukami *et al.*[319] developed a C_{60} solublising solid-solid mechanochemical procedure involving cycloamyloses, which avoids the need for organic solvents. Deguchi *et al.* [320] found that fullerene C60 particles size as small as 20 nm are readily produced by grinding the bulk solid, which can be dispersed in water with or without the aid of a surfactant such as sodium dodecylsulfate.

Under specific conditions *p*-sulfonatocalix[4]arene is effective in solubilizing fullerenes C_{60} and C_{70} in water. This is despite the well known lack of complementarity of size and shape of the cavity of this calixarene relative to the fullerenes. It is calix[5]arenes and higher calixarenes that can form such host-guest complexes, with calix[4]arenes restricted to binding small molecules such as solvent and gas molecules.

12.2 Materials and methods

Fullerenes C_{60} and C_{70} were purchased from Aldrich and Fluka with an as-received purity of 99%, and were used without further purification. *N*-methylimidazole, 1,3-propane sulfone and 1,4-butane sultone were purchased from Aldrich and used as received. Sodium *p*-sulfonatocalix[4]arene was synthesized described in Section 3.4.

Filtered samples (through a membrane filter of 0.2 μm pores) were characterized using Shimadzu UV-2500PC spectrometer within 10-mm quartz cuvettes with spectral range of 900 to 250 nm. Transmission Electron Microscope (TEM) images were obtained from samples which were prepared by drop cast of dispersed nanocomposite on copper grid carbon film (300 mesh), with Leo Libra 120 microscope operating at an accelerating voltage of 120 kV. Dynamic Light-Scattering (DLS) was performed using Malvern Zetasizer Nanoseries. ^{1}H NMR spectra were collected on a Varian 400 MHz spectrometer using D_2O at 25^{o}C.

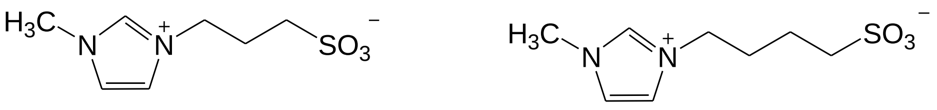
12.2.1 Synthesis of Brønsted acidic ionic liquids

1-(4-sulfonic acid) propyl-3-methylimidazolium (1)

1,3-Propane sulfone (0.1 mol) was dissolved in acetate ether and stirred vigorously. 1-Methylimidazole (0.1 mol) was dropped slowly in the solution at 50 ^{o}C. The mixture was stirred continously for 2 hours, and the resultant mixture was filtered to obtain white precipitate. The precipitate was then washed with acetate ether several times and dried to get white powder. The product was characterised by ^{1}H NMR (300MHz, D_2O) δ: 8.7 (s, 1H), 7.5 (s, 1H), 7.4 (s, 1H), 4.3 (t, 2H), 3.8 (s, 3 H), 2.8 (t, 2H), 2.3 (m, 2H).

1-(4-sulfonic acid) butyl-3-methylimidazolium (2)

N-Methylimidazole, toluene, and 1,4-butane sultone were placed in a dry round-bottomed flask fitted with a reflux cooler and a mechanical agitator. The mixture was vigorously stirred at 80 ^{o}C for 8 hours and then a white solid was filtered and dried. The product was characterised by ^{1}H NMR (300MHz, D_2O) δ: 8.6 (s, 1H), 7.3 (s, 1H), 7.2 (s, 1H), 4.1 (m, 2H), 3.7 (d, 3H), 2.7 (m, 2H), 1.8 (m, 2H), 1.6 (m, 2H).



(**1**) (**2**)

Figure 12.1. Structure of Brønsted acidic ionic liquid (**1**) and (**2**)

12.2.2 Synthesis of water soluble *p*-sulfonatocalix[4]arene/fullerenes C_{60} and C_{70}

The synthesis method of the calixarene/fullerene composite material was carried out by mixing 10:1 molar ratios of *p*-sulfonatocalix[4]arene to fullerene, (ca 2.12 and 2.52 mg of fullerene C_{60} and C_{70} respectively), using mechanical grinding in a mortar and pestle for 20 minutes, followed by the addition of 5 mL of deionized water then further grinding 5 minutes. The mixture was ultrasonicated (Elmasonic S30H, frequency: 37 kHz, power: 320 W) for 30 minutes then centrifuged (Kubota 2420, 4000rpm) for 30 minutes, affording coloured supernatant. The yield percentage of the isolated solid at the bottom of the tube on removing the water is approximately 4% to 6%.

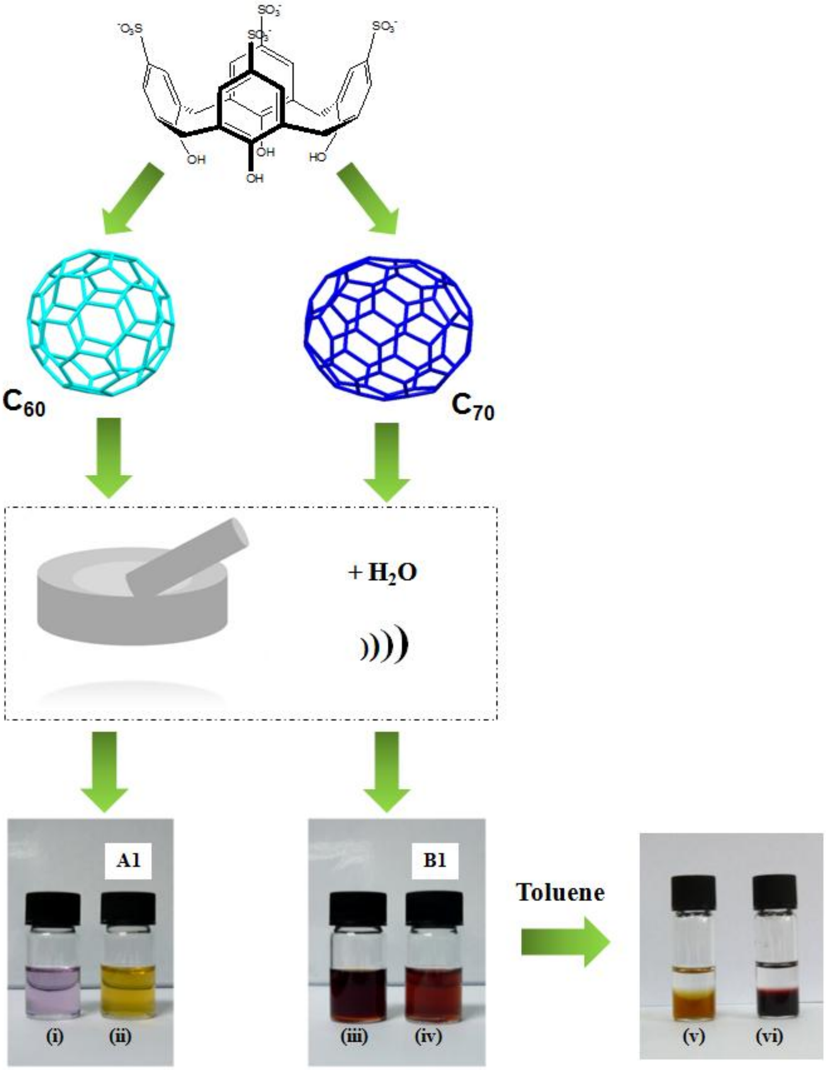


Figure 12.2. Preparation of water-soluble fullerene nano-composites.

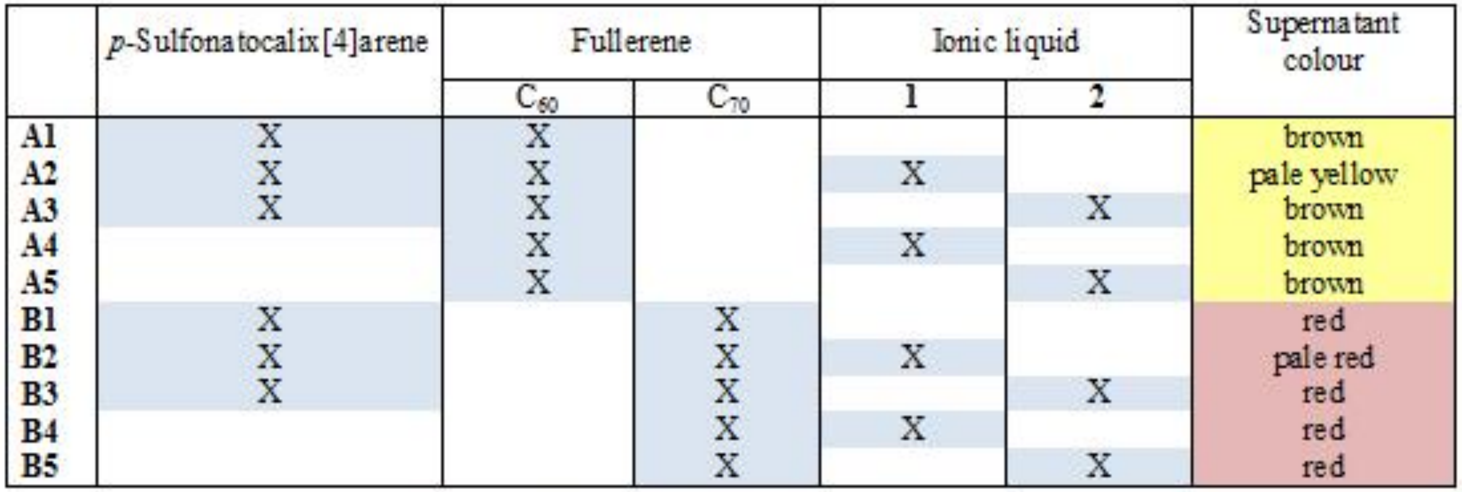
12.2.3 Synthesis of *p*-sulfonatocalix[4]arene/ionic liquid/fullerenes C_{60} and C_{70}.

Equimolar of *p*-sulfonatocalix[4]arene and Brønsted acidic ionic liquid (**1** or **2**) were dissolved in water and stirred continously for 4 to 5 hours. Water was removed under reduced pressure to yield beige solids. The solid was mixed with fullerene with 10:1 molar ratios, using mechanical grinding in a mortar and pestle for 20 minutes, followed by the addition of 5 mL of deionized water then further grinding 5 minutes. The mixture was ultrasonicated (Elmasonic S30H, frequency: 37 kHz, power: 320 W) for 30 minutes then centrifuged (Kubota 2420, 4000rpm) for 30 minutes, affording coloured supernatant. The yield percentage of the isolated solid at the bottom of the tube on removing the water is approximately 6% to 7%.

12.2.4 Synthesis of ionic liquid/fullerenes C_{60} and C_{70}

Ionic liquid/fullerene composite material was carried out by mixing 10:1 molar ratios of (**1**) or (**2**) to fullerene, using mechanical grinding in a mortar and pestle for 20 minutes, followed by the addition of 5 mL of deionized water then further grinding 5 minutes. The mixture was ultrasonicated (Elmasonic S30H, frequency: 37 kHz, power: 320 W) for 30 minutes then centrifuged (Kubota 2420, 4000rpm) for 30 minutes, affording coloured supernatant. The yield percentage of the isolated solid at the bottom of the tube on removing the water is approximately 8% to 10%.

Table 12.1: Summary of components affording water-soluble fullerene nano-composite materials.



	p-Sulfonatocalix[4]arene	Fullerene		Ionic liquid		Supernatant colour
		C_{60}	C_{70}	**1**	**2**	
A1	X	X				brown
A2	X	X		X		pale yellow
A3	X	X			X	brown
A4		X		X		brown
A5		X			X	brown
B1	X		X			red
B2	X		X	X		pale red
B3	X		X		X	red
B4			X	X		red
B5			X		X	red

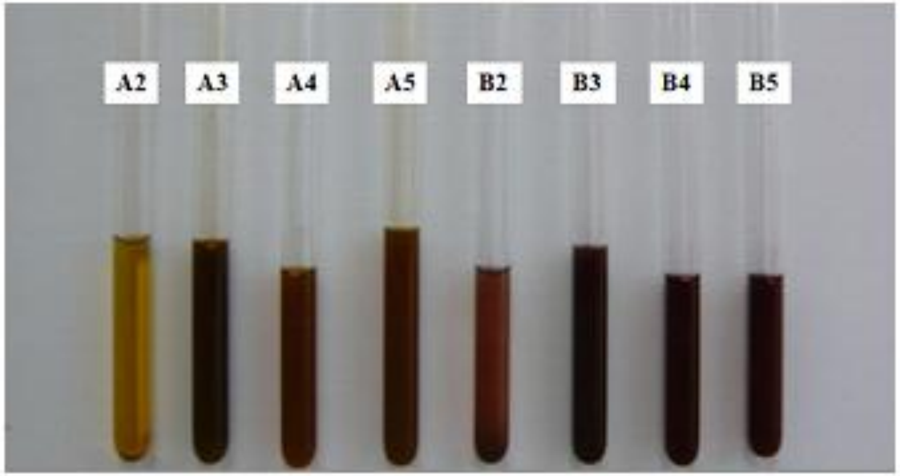


Figure 12.3. Aqueous solutions of samples **A2** to **B5**.

12.3 Results and discussion

The method of solubilising the fullerenes in water involves mechanical grinding and ultrasonication in water. The transformation from magenta solutions for C_{60} dissolved in toluene to yellow for the *p*-sulfonatocalix[4]arene/C_{60} composite material in water relates to the formation of discrete solvated fullerenes in toluene [321] versus aggregation of the fullerenes in water. While red solution was detected for *p*-sulfonatocalix[4]arene/C_{70} dissolved in water relative to the composite material and this is in agreement with the report on fullerene with calix[5]arene [322].

Interestingly, the aqueous solutions are stable over several months, and are stable in the presence of toluene with vigorous mixing with no dissolution in the organic phase. This is consistent with the presence of nano-particles of the fullerene surrounded by a sheath of the sulfonated calixarene, forming a protective layer. This type of protection has been noted for carotenoid nanoparticles stabilised by selected sulfonated calixarenes[63].

12.3.1Electronic absorption spectroscopy

A common method to investigate interplay of cavitands and fullerenes, and aggregation of fullerenes involves the use of UV-visible spectroscopy, with the region between 400 to 650 nm assigned to symmetry-forbidden electronic transition and the symmetry-allowed vibronic transitions of the fullerenes [323]. Solvated monomeric C_{60} in toluene gives magenta solutions, with a characteristic peak at $\lambda = 408$ nm. Ágnes Buvári-Barcza reported cyclodextrin-C_{60} complexes in water give yellowish-brown solutions containing aggregates of the C_{60} molecules surrounded by cyclodextrin molecules with no peak at $\lambda = 408$ nm [324].

A characteristic absorption peak of C_{60} at $\lambda = 404–408$ nm was observed whereas complexes of *p*-sulfonatocalix[4]arene-C_{60} (**A1**) and *p*-sulfonatocalix[4]arene/sulfonato-imidazolium/C_{60} (**A2** and **A3**) showed significant broadening of the absorption band in the visible region with maximum absorption at ~350 to 360 nm and was found shifted as compared to pristine C_{60}. Complexes of ionic liquid-C_{60} (**A4** and **A5**) showed weaker absorption intensities relative to the other complexes. Another key change in the electronic spectrum of **A1**, **A2** and **A3** relative to that of the fullerene in toluene is the appearance of a band at 450 nm which corresponds to resonant inter-fullerene molecular transitions and thus the presence of aggregates of C_{60} [323, 325]. However this band is not present in samples **A4** and **A5**. Presumably, *p*-sulfonatocalix[4]arene leads the growth of fullerene clustering or fullerene aggregation.

The characteristics of the C_{70} monomer absorption in the visible region are the maximum absorption at ~470 nm with a small shoulder at ~550 nm and a strong narrow absorption peak at ~380 nm. For *p*-sulfonatocalix[4]arene-C_{70} (**B1**) two peaks in the ~370 to 600 nm were observed whereas for *p*-sulfonatocalix[4]arene/sulfonato-imidazolium/C_{70} complexes (**B2** and **B3**) have two similar absorption bands in the same region however with the intensities decreased. Ionic liquid-C_{60} complexes (**A4** and **A5**) have very low absorption in their spectra.

The spectral changes in the UV-visible spectra indicate possible complex interaction between the water-soluble calixarene and fullerenes (C_{60} and C_{70}), either with or without the presence of sulfonato-imidazolium, which presumably forms stable water-soluble aggregates. It has been published elsewhere that there is distinct difference observed between C_{60} and C_{70} aggregates in relation to their absorption spectra. For C_{60} aggregation, a blue shift is observed in the absorption maxima in comparison to its monomer spectra [326, 327] whereas for C_{70} the aggregation causes a red shift in the absorption maxima. These observations indicate that the mode of packing of the fullerene molecules in the aggregates is be different for the two fullerene analogues where the blue shift in the absorption maxima for C_{60} shows that the stacking of the monomers in the aggregates results in the formation of H-type of aggregation while a red shift for C_{70} aggregation indicates that the monomers stacking results in the J-type of aggregation [328, 329]. This observation could be related to the difference in the shape of two fullerene molecules, spherical for C_{60} and ellipsoidal for C_{70} [330].

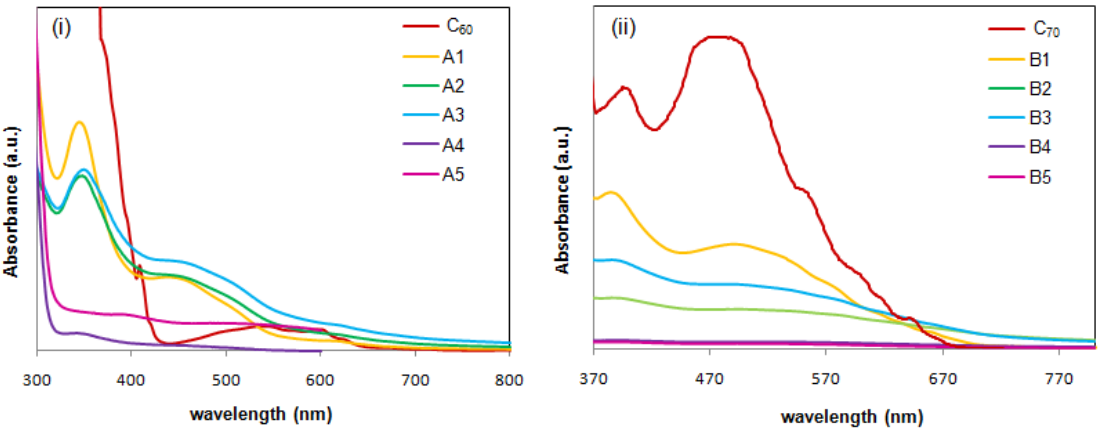


Figure 12.4. UV-visible spectra of (i) C_{60} (in toluene) and C_{60} nano-composites (in water); **A1** to **A5** and C_{70} (in toluene) and C_{70} nano-composites (in water); **B1** to **B5**.

12.3.2 Solution studies

NMR spectra for *p*-sulfonatocalix[4]arene-C_{60} (**A1**) and *p*-sulfonatocalix[4]arene-C_{70} (**B1**) showed showed calixarene are in various distorted cone conformations with the appearance of a broad peak in the 3.0 to 4.5 ppm region, however for *p*-sulfonatocalix[4]arene/sulfonato-imidazolium/C_{60} complexes (**A2** and **A3**) and *p*-sulfonatocalix[4]arene/sulfonato-imidazolium/C_{70} complexes (**B2** and **B3**), the calixarene methylene protons are resolved which indicate that the calixarenes are predominantly all in the same cone conformation. The presence of sulfonato-imidazolium, **1** or **2**, in the *p*-sulfonatocalix[4]arene–fullerene composites for samples **A2** and **B2** or **A3** and **B3** respectively have chemical shifts differences relative to the sulfonato- imidazolium, with the most significant shifts experienced by the imidazolium ring protons while the aliphatic protons have the least chemical shifts. This observation is consistent with the solution studies of calixarene–imidazolium cation supermolecules in aqueous solution where the imidazolium charge head group is selectively drawn into the calixarene cavity. The NMR results suggest the possibility of calixarene and sulfonato-imidazolium supermolecules decorating the fullerene molecules with the sulfonate tail of the sulfonato-imidazolium and the sulfonate groups of calixarene directed into the water environment.

For samples **A4**, **A5**, **B4** and **B5**, upfield chemical shifts were detected with the largest shift experienced by the protons of the methyl terminal, followed by the aromatic protons and the aliphatic protons experienced the least shift changes indicating the molecules have interactions between themselves or with fullerene.

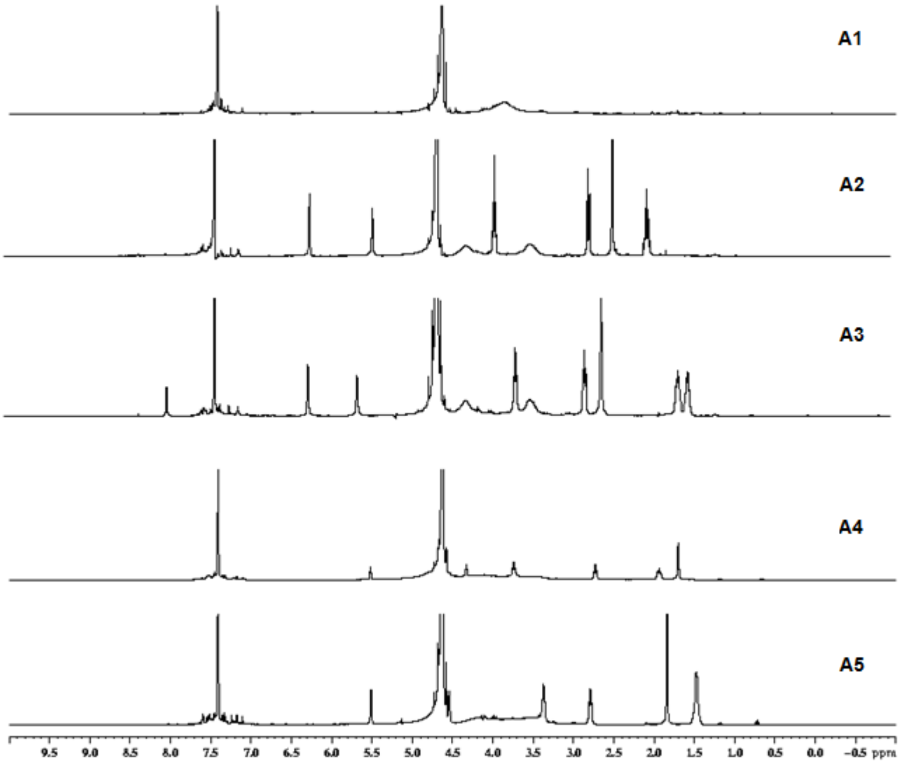


Figure 12.5. ^{1}H NMR spectra of C_{60} nano-composites (in D_2O, 25^{o}C).

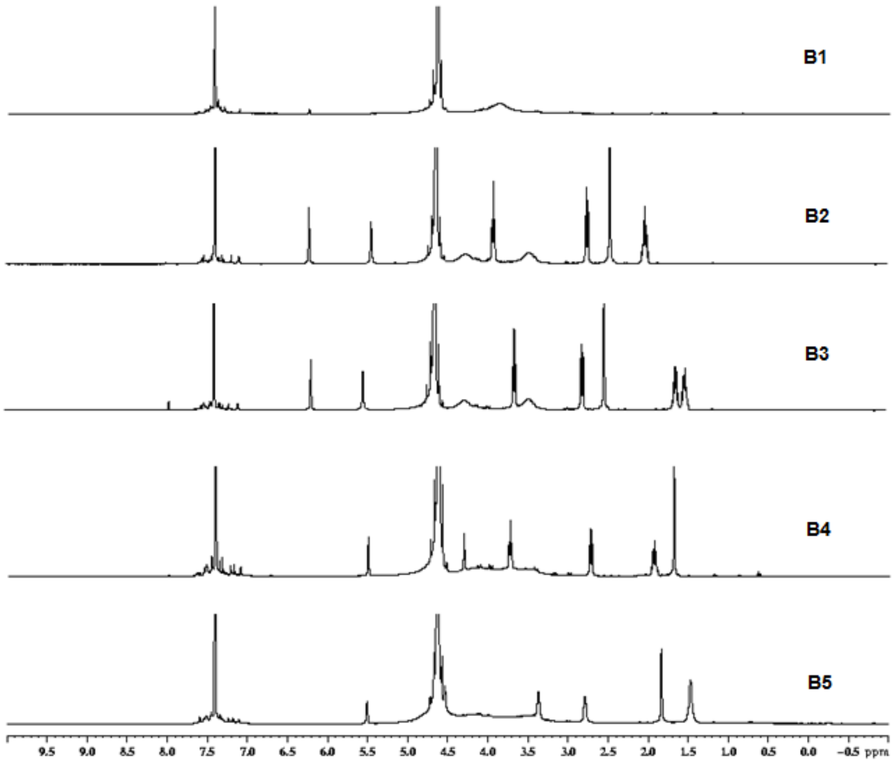


Figure 12.6. ^{1}H NMR spectra of C_{70} nano-composites (in D_2O, 25^{o}C).

12.3.3 Particle size measurement

TEM photoimages of all complexes **A1** to **A5** and **B1** to **B5** are shown in Figure 12.9 and 12.10. The nano-composite materials are fairly monodisperse in size and are observed in an aggregated state with S element surrounding the nano-composite particles as illustrated in the element mapping. The aggregation could have taken place in the specimen preparation process and most probably during drying. The presence of such discrete non-uniform spherical particles was reminiscent of typical 'raspberry morphology' as previously reported by Ballav [331]. Samples **A1** and **B1** formed discrete spherical particles with sizes of average diameter varied from 30 to 50 nm, which are comparable to the particle sizes determined by DLS measurements (average particle size: 30 to 60 nm). Larger spherical particles were observed when ionic liquids component were incorporated into the samples (**A2**, **A3**, **B2** and **B3**) where the average diameter varied from 40 to 100 nm for fullerene C_{60} and C_{70}, while in absence of calixarene (**A4**, **A5**, **B4** and **B5**) the average diameter varied from 60 to 110 nm where the shape of the particles appeared to be irregular. Negative Zeta potentials for all aqueous samples were obtained and higher zeta potentials were found for complexes containing calixarene which suggests that the complexes confer higher electrically stability.

Table 12.2: Summary of particle sizes measured using TEM and the Zeta potentials for the fullerene nano-composites.

	TEM: Particle size, nm	**Zeta Potential, mV**
A1	40.3 ± 7.3	-55.9 ± 14.6
A2	90.4 ± 4.6	-53.0 ± 19.0
A3	50.5 ± 4.2	-55.4 ± 15.2
A4	60.3 ± 4.5	-37.0 ± 19.3
A5	100.2 ± 5.5	-39.1 ± 16.7
B1	51.5 ± 6.5	-53.3 ± 17.5
B2	85.6 ± 6.2	-55.5 ± 14.3
B3	62.3 ± 8.6	-51.7 ± 15.3
B4	126.3 ± 9.8	-33.0 ± 16.6
B5	88.3 ± 15.8	-37.5 ± 16.0

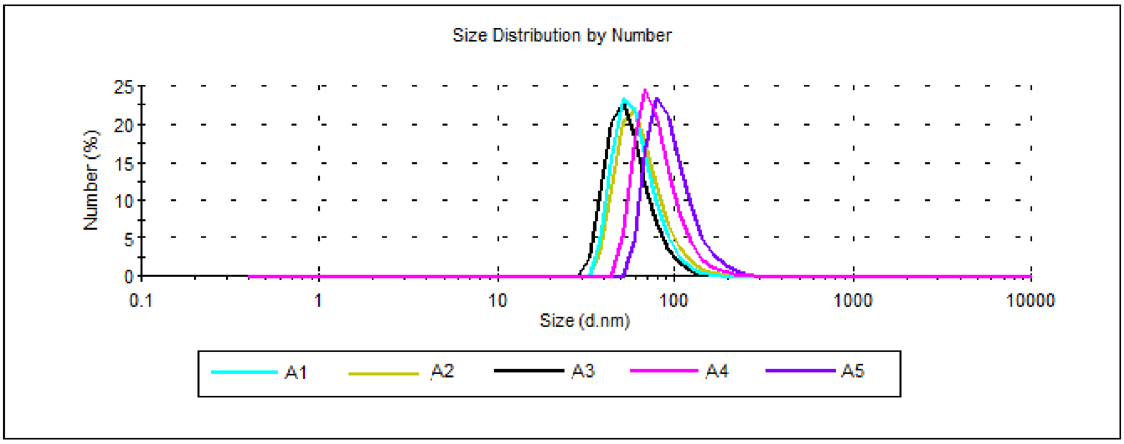


Figure 12.7. Particle size distribution graphs measured using DLS for water-soluble fullerene C_{60} nano-composites.

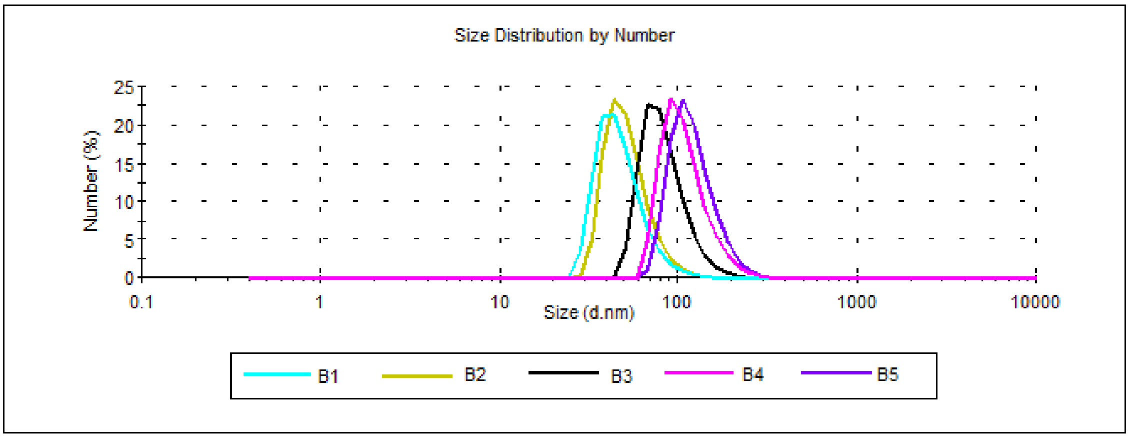


Figure 12.8. Particle size distribution graphs measured using DLS for water-soluble fullerene C_{70} nano-composites.

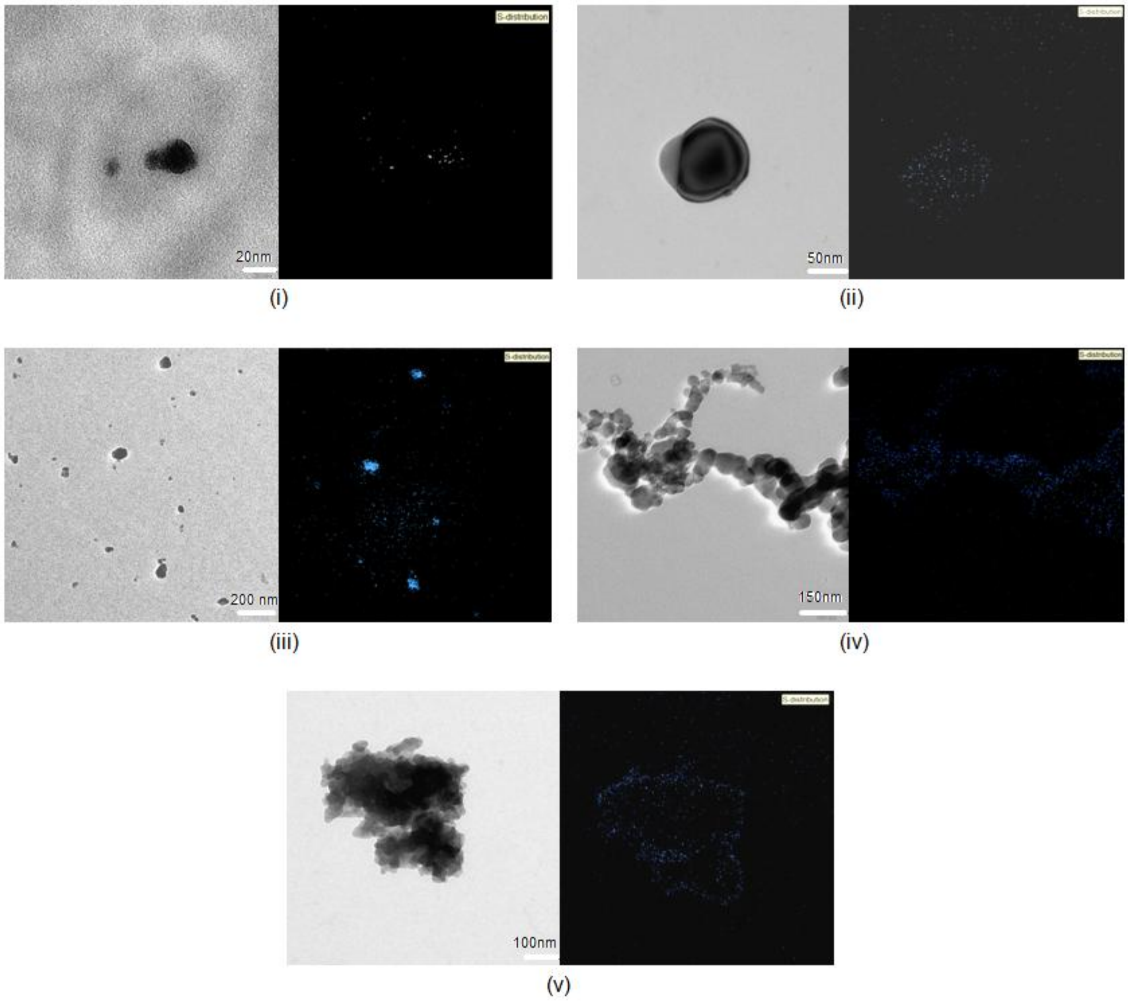
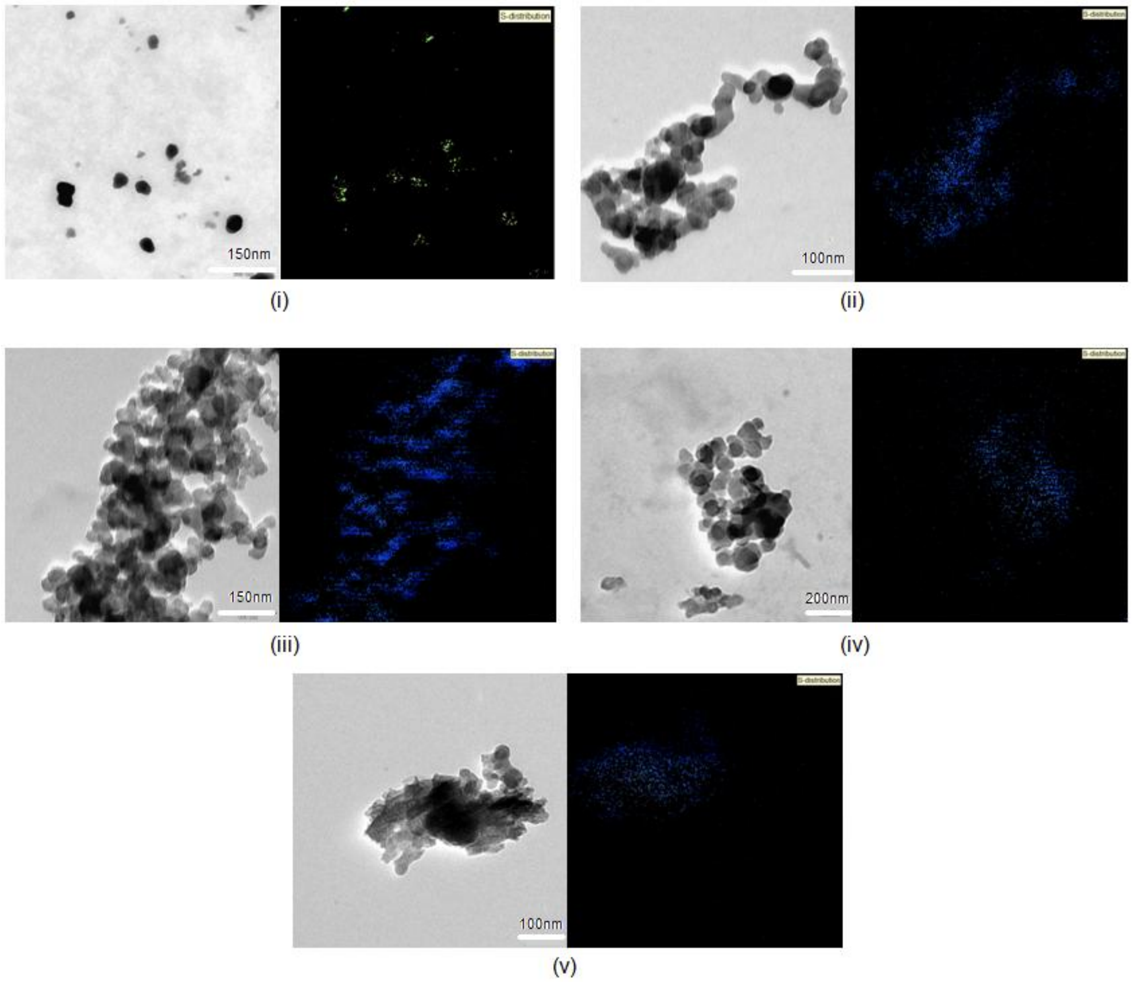


Figure 12.9. TEM images showing nano-particles for (i) **A1**, (ii) **A2**, (iii) **A3**, (iv) **A4** and (v) **A5** with the corresponding sulfur element mapping.



(i) (ii)

(iii) (iv)

(v)

Figure 12.10. TEM images showing nano-particles for (i) **B1**, (ii) **B2**, (iii) **B3**, (iv) **B4** and (v) **B5** with the corresponding sufhur element mapping.

12.4 Conclusions

Fullerenes can be solubilised in water by mechanical grinding with *p*-sulfonatocalix[4]arene and/or sulfonato-imidazolium, followed by sonication in water. The composite nanomaterial has nano-particles of the calixarene or calixarene-ionic liquid supermolecules arranged on the surface of the fullerene. Even the stability of the fullerene nano-particles with respect to being taken up in toluene, the outer layer of the nano-particles of fullerene are assembled in a compact, impenetrable layer, possibly as the ubiquitous bilayer arrangement with prevails for crystalline material based on the self assembly of the calixarene.