Chapter 5
Solubilization of Fullerenes
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5.1 Solubilization of [60]Fullerene by Modified β-Cyclodextrin

5.1.1 Introduction

Buckminsterfullerene C_{60}, the third carbon allotrope has been the subject of many interesting applications during the last two and half decades because of its unique physical, chemical and biological properties.\textsuperscript{1-5} The continuing interest in this molecule is evident from the number of papers that appear every year in the literature.\textsuperscript{6-9} This is due to its biological activity including DNA-cleaving ability, radical scavenging, MRI enhancement and anti-HIV activity.\textsuperscript{10-13} A number of such biological applications emerge from the fullerenes behaving as an efficient radical scavenger and known as ‘free radical sponge’. However, the potential biomedical applications of fullerenes are hindered due to its hydrophobicity and biomedical applications of fullerenes require genuine water-solubility with no agglomeration. The first report on aqueous solubilization of [60]fullerene with cyclodextrins was described by Andersson et al\textsuperscript{14} where [60]fullerene was taken into alcoholic boiling aqueous solution with γ-CD. The authors concluded that due to the inner cavity size of 0.950 nm, only γ-CD can form inclusion complex with [60]fullerene and α-CD and β-CD with inner cavity size of 0.57 nm and 0.78 nm respectively cannot form inclusion complex with [60]fullerene having a 1 nm diameter. Subsequently, Murthy et al\textsuperscript{15} showed that not only γ-CD but also β-CD can form inclusion complex with [60]fullerene given the right reaction conditions arguing that β-CD can form 2:1 inclusion complex with fullerene. After that several methods have been developed and described for the preparation of water-soluble fullerenes by forming complexes with water-soluble calix[n]arenes,\textsuperscript{16,17} polyvinyl alcohols,\textsuperscript{18} and polyvinyl pyrrolidone\textsuperscript{19,20}, however cyclodextrins being naturally occurring and water-soluble seem to be the ideal hosts and has been comprehensively investigated for aqueous solubilization of fullerenes for biomedical applications.

The unique ability of cyclodextrins is that they can encapsulate the [60]fullerene molecule (depending upon the size of the non-polar inner cavity) non-covalently into the non-polar cavity and make it water-soluble\textsuperscript{14,15}. However, a
competitive guest molecule in the biological media can displace [60]fullerene from the CDs cavity, thus defeating the purpose of using CDs for encapsulating [60]fullerene. Therefore covalently linked fullerene with CDs would be a better solution to overcome this problem. The high chemical reactivity of the fullerene molecules allows the synthesis of an enormous number of fullerene derivatives for biocompatible materials and biological applications\textsuperscript{21}. The reaction of [60]fullerene with azides under elevated temperature has been well studied in literature. The azides are known to add to C\textsubscript{60} via cycloaddition reaction by forming triazoline intermediate, which then decompose to yield the desired product by elimination of N\textsubscript{2}, or by the formation of a nitrene, that adds to C\textsubscript{60} double bond. It has been found to be an efficient process resulting in fullerene derivatives containing an expanded cage of fullerene as azafullerenes C\textsubscript{60}N\textsuperscript{22}.

The synthesis of water-soluble β-CD-[60]fullerene adduct by adopting supramolecular covalent as well as non-covalent interactions of [60]fullerene with selectively diazide functionalized β-CD is described.
5.1.2 Experimental

5.1.2.1 Materials

[60] Fullerene purity (99.9%) was purchased from Sigma-Aldrich. Dimethylsulfoxide (DMSO) and toluene were purchased from Merck (India) and dried over calcium hydride for 24h and then distilled under reduced pressure before use. Diazido β-cyclodextrin synthesized in Chapter 2 was used. Double distilled water was used during the experimental work.

5.1.2.2 Measurements

The FTIR spectra of the compounds were recorded on a Shimadzu 8400S Fourier Transform Infrared Spectrometer by KBr pellet method at 10⁻⁴ resolution and 30 scans at room temperature. AR grade KBr was used for the preparation of pellet. The UV-Vis spectra were recorded on a Shimadzu UV-Vis-2450 spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Bruker spectrometer operating at 400 MHz at room temperature where TMS was used as internal standard. Thermogravimetry analysis (TGA) was recorded on a Shimadzu, TGA-50 system with a heating rate of 10⁰C/min under air atmosphere in the temperature range of 30-800⁰C. XRD patterns were measured on a Rikagu diffractometer with CuKα radiation (λ = 0.15406 nm) at 40 kV and 40 mA. To study the morphology and particle size of adduct, an energy-filtering transmission electron microscopy (TEM) [EF-TEM, EM 912 OMEGA (ZEISS, S-4700), 120 kV] was used. For TEM observation, the 1% adduct solution in double distilled water was drop casted on 400 mesh carbon-coated copper grids and annealed at 100⁰C for 12 hours before measurement. The measurements of scanning electron microscopy of powders on the carbon tape after gold-coating were performed on a JEOL JSM-6500F at an operation voltage of 20 kV. AFM measurements were carried out on a Nanoscope III (Digital Instruments) in air. The D₂O solution of adduct was deposited on a mica plate and the D₂O was evaporated under reduced pressure. Tapping mode analysis was carried out on the plate.
5.1.2.3 Synthesis of β-Cyclodextrin-[60]Fullerene Adduct

The water-soluble β-cyclodextrin-[60]fullerene adduct as shown in Scheme 5.1.1 was synthesized using a mixed solvent system to bring water-soluble and toluene soluble reactants into one homogeneous phase. Thus, a 0.1 mmol solution of 6\textsuperscript{A},6\textsuperscript{D}-diazido-6\textsuperscript{A},6\textsuperscript{D}-dideoxy-β-cyclodextrin in DMSO and a 0.1 mmol solution of [60]fullerene in toluene were mixed and stirred for 36h at 80°C under nitrogen atmosphere, during which the deep purple homogeneous solution turned to deep brown. The progress of the reaction was monitored by withdrawing aliquots of the reactant solution in intervals of 4h and measuring the UV-Vis absorbance. After the above mentioned time period, the product formed was isolated by removing the organic solvents under vacuum on a rotary evaporator. The brown colored solid was extremely water-soluble giving a light yellow aqueous solution. This aqueous solution was filtered through 0.45 μm syringe-filter to remove undissolved residue and then the filtrate was subjected to ultrafiltration over a polymer membrane with a molar mass cut-off of 2 kg mol\textsuperscript{-1} for the removal of excess of diazido β-cyclodextrin. The resultant concentrated aqueous solution was freeze-dried to get a deep brown colored adduct, which is soluble in water.

The 2:1 inclusion complex of β-cyclodextrin and [60]fullerene was prepared according to the literature\textsuperscript{15} and adopting the same procedure as shown in Scheme 5.1.2 for the comparison studies.
Scheme 5.1.1 Covalent and Non-covalent interaction of modified β-CD and [60]fullerene.

Scheme 5.1.2 Non-covalent 2:1 inclusion complex of β-CD and [60]fullerene.
5.1.3 Results and Discussion

The solubility of the β-CD-[60]fullerene adduct in water was measured and found to be 6.5 mg mL\(^{-1}\), which is greater than that of 2:1 inclusion complex of β-cyclodextrin\(^9\). Usually, apart from the solubility of fullerenes in aqueous solution, the stability of the aqueous solution of fullerenes is a major problem, owing to their tendency to agglomerate after storage for longer periods. Hence, the stabilization of aqueous solution of solubilized fullerenes with higher stability is a pivotal issue and, therefore, is a challenge to produce aqueous solution of fullerenes with higher stability to explore their potential in biomedical applications. The previously described methods for the aqueous solubilization only deal with the non-polar interaction of the [60]fullerene molecule with cyclodextrins\(^{14,15}\). However, a competitive guest molecule in the biological media can displace [60]fullerene from the cyclodextrin cavity. Tethering the [60]fullerene molecule to the improved water-soluble selectively functionalized cyclodextrin molecule is an effective option of overcoming this problem. It also takes into account the advantage of more [60]fullerene surface area, which is not in the case of 2:1 inclusion complex. The β-CD-[60]fullerene adduct was stable and fullerene was not extractable from this complex.

To see the visible evidence of covalent-noncovalent interactions and stability of the β-CD-[60]fullerene adduct in polar and non-polar solvents, the extraction experiment was conducted in vials. [60]Fullerene in toluene shows deep purple color (Figure 5.1.1 [A]) and it is completely insoluble in polar solvents. Diazido β-CD is completely soluble in polar DMSO (Figure 5.1.1 [B]). These two solutions when mixed together in inert atmosphere and after evaporation of organic solvents, resulted in a yellowish brown color water-soluble β-CD-[60]fullerene adduct (Figure 5.1.1 [C]). When toluene was added to the solid β-CD-[60]fullerene adduct (Figure 5.1.1 [D]), it was observed that β-CD-[60]fullerene adduct was stable enough and [60]fullerene does not go into the toluene layer and it settled at the bottom of the vial. When toluene was added to the aqueous solution of β-CD-[60]fullerene adduct (Figure 5.1.1 [E]), it was observed that aqueous solution of β-CD-[60]fullerene adduct was stable enough and [60]fullerene does not go into the toluene layer. Thus, the β-CD-[60]fullerene adduct was stable in both the polar and non-polar solutions,
whereas, this is completely inverted in the case of 2:1 inclusion complex of β-CD with [60]fullerene. When toluene was added to the solid β-CD-[60]fullerene 2:1 inclusion complex (Figure 5.1.2 [D]), it was observed that [60]fullerene can easily go into the toluene solution and also similar observation has been seen when toluene was added to the aqueous solution of β-CD-[60]fullerene 2:1 inclusion complex (Figure 5.1.2 [E]), since toluene can penetrate into the non-polar cavity of β-CD and act as competitive guest molecule it can displace [60]fullerene from the non-polar cavity of β-CD and itself occupy into the non-polar cavity of β-CD.

**Figure 5.1.1** Photograph of [A] C$_{60}$ in toluene, [B] Diazido β-CD in DMSO, [C] β-CD-C$_{60}$ adduct in water, [D] β-CD-C$_{60}$ adduct in toluene and [E] β-CD-C$_{60}$ adduct in water + toluene.

**Figure 5.1.2** Photograph of [A] C$_{60}$ in toluene, [B] β-CD in DMSO, [C] β-CD-C$_{60}$ inclusion complex in water, [D] β-CD-C$_{60}$ inclusion complex in toluene and [E] β-CD-C$_{60}$ inclusion complex in water + toluene.
The more specific evidence for the covalent as well as non-covalent interactions between selectively diazido functionalized β-cyclodextrin and [60]fullerene comes from the conventional techniques like FTIR, UV-Vis, $^1$H NMR, $^{13}$C NMR and TGA of the β-CD-fullerene adduct.

### 5.1.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of diazido β-CD, $C_{60}$ and β-CD-[60]fullerene adduct is depicted in Figure 5.1.3. The FTIR spectrum of the diazido functionalized β-CD (Figure 5.1.3 a) showed an absorbance stretching band at 2037 cm$^{-1}$ for asymmetrical azide (-N$_3$) functionality along with all the absorbance bands of the parent β-cyclodextrin, whereas there was no signal in this region in the FTIR spectrum (Figure 5.1.3 c) of the β-CD-[60]fullerene adduct. The spectrum of pristine $C_{60}$ (Figure 5.1.3 b) showed four characteristic absorption bands at 1429 cm$^{-1}$, 1183 cm$^{-1}$, 578 cm$^{-1}$ and 527 cm$^{-1}$. The β-CD-[60]fullerene adduct showed the typical sharp peaks of [60]fullerene at 527 cm$^{-1}$ and 583 cm$^{-1}$. The disappearance of absorbance band of azide (-N$_3$) and presence of [60]fullerene absorbance bands confirmed the covalent attachment of fullerene with modified β-CD.

![FTIR spectra](image)

**Figure 5.1.3** FTIR spectra of (a) diazido β-CD, (b) $C_{60}$, (c) β-CD-C$_{60}$ adduct.
5.1.3.2 UV-Visible Spectroscopy (UV-Vis)

The UV-Vis absorbance spectra of C\textsubscript{60} in toluene, diazido β-CD in water and β-CD-[60]fullerene adduct in water is shown in Figure 5.1.4. The UV-Vis absorbance spectrum of [60]fullerene showed absorbance maxima at around 334 nm in toluene and it is completely insoluble in water, whereas the diazido β-cyclodextrin does not show any absorbance in this region. However, the spectrum of β-CD-fullerene adduct in water showed the typical absorbance maxima at around 348 nm of [60]fullerene which is red shifted by few nanometers and there was peak broadening of the absorbance beyond 406 nm typical of the [60]fullerene absorbance in toluene.

![UV-Vis Spectra](image)

**Figure 5.1.4** UV-Vis spectra of C\textsubscript{60} in toluene, diazido β-CD in water and β-CD-C\textsubscript{60} adduct in toluene.

The supramolecular interaction of [60]fullerene with the modified β-CD was confirmed by extracting [60]fullerene with a competitive guest molecule. Thus, when toluene was added (toluene acts as competitive guest molecule and also solvent for fullerene) to the aqueous solution of the modified β-CD-[60]fullerene adduct as well...
as to a 2:1 inclusion complex of β-CD/[60]fullerene and shaken, the toluene layer was characterized by UV-Vis spectroscopy, it showed that 2:1 inclusion complex of β-CD/[60]fullerene shows an absorbance at 335 nm of [60]fullerene (Figure 5.1.5) whereas the toluene layer of the modified β-CD-[60]fullerene adduct did not show any absorbance. This indicates that the [60]fullerene was covalently attached to modified β-CD and also forms an inclusion complex with another molecule of modified β-CD. This behavior proved that in a competitive milieu the modified β-CD-[60]fullerene adduct is more stable than the 2:1 inclusion complex of β-CD/[60]fullerene.

![UV-Vis spectra of C₆₀ in toluene, β-CD-C₆₀ 2:1 inclusion complex in water and β-CD-C₆₀ 2:1 inclusion complex in toluene.](image)

**Figure 5.1.5** UV-Vis spectra of C₆₀ in toluene, β-CD-C₆₀ 2:1 inclusion complex in water and β-CD-C₆₀ 2:1 inclusion complex in toluene.

5.1.3.3 Nuclear Magnetic Resonance Spectroscopy (NMR)

The type of interactions between [60]fullerene and modified β-cyclodextrin was further confirmed by ¹H and ¹³C NMR. The ¹H NMR spectra of the diazide functionalized β-CD and β-CD-[60]fullerene adduct was taken in two different
solvents DMSO-d$_6$ and D$_2$O shown in Figure 5.1.6 and Figure 5.1.7, respectively. The $^1$H NMR spectra of diazido β-CD and adduct shows the similar proton signal due to the similar hydrogen structure, except upfield shifts was observed. The $^1$H NMR spectra given in Figure 5.1.6 of the β-CD-[60]fullerene adduct showed all the proton signals of the glucose units of the cyclodextrin at δppm: 4.81 (d, 7H, H-1), 3.27 (m, 7H, H-2), 3.66 (m, 7H, H-3), 3.36 (m, 7H, H-4) 3.56 (m, 7H, H-5), 3.63 (d, 12H, H-6) and it was observed that the proton signals in adduct shows upfield shifts compared to the diazido β-CD, which confirms the formation of inclusion complex. The spectra was similar when it was taken in a different solvent i.e. D$_2$O with no observable shifts in the positions of the peaks.

![Diagram of β-CD-C$_{60}$ adduct](image1)

![Diagram of Diazido β-CD](image2)

Figure 5.1.6 $^1$H NMR spectra of diazido β-CD and β-CD-C$_{60}$ adduct in DMSO-d$_6$. 

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Figure 5.1.7 $^1$H-NMR spectra of diazido $\beta$-CD and $\beta$-CD-$C_{60}$ adduct in D$_2$O.

The $^{13}$C NMR spectra of $\beta$-CD-$[60]$fullerene adduct in two different solvents D$_2$O and DMSO-d$_6$ is shown in Figure 5.1.8. The $^{13}$C NMR spectra of the $\beta$-CD-$[60]$fullerene adduct in both the solvents showed all the expected six carbon chemical shift of glucose units at $\delta$ppm: 101.85 (C-1), 72.42 (C-2), 73.04 (C-3), 82.29 (C-4), 72.26 (C-5), 6.27 (C-6) along with three new carbon chemical shifts. The one at 32 $\delta$ppm is due to the primary substituted carbon (C-6') of $\beta$-CD and the other two at 70.7 $\delta$ppm and at 165 $\delta$ppm are due to the formation of [5,6] closed azafulerene structure with $\beta$-CD. It is interesting to note that the $^{13}$C NMR is clean with new peaks confirming the formation of covalent bonds as determined by reaction between olefinic double bond and an azide. The spectra in both the solvents look similar due to the same structural carbon skeleton.
**Figure 5.1.8** $^{13}$C-NMR spectrum of $\beta$-CD-$C_{60}$ adduct in D$_2$O and DMSO-$d_6$.

### 5.1.3.4 Thermogravimetric Analysis (TGA)

In order to assess the thermal stability of the $\beta$-cyclodextrin derivative and $\beta$-CD-$[60]$fullerene adduct, a thermogravimetric study was conducted. The degradation pattern of parent cyclodextrin shows mass loss in three different temperature regions (Figure 5.1.9 a). The first mass loss at around 105°C is due to the loss of moisture, the second mass loss at 300°C as dehydration of $\beta$-CD, and third mass loss at 367°C as a decomposition of glucose units in $\beta$-CD. However, in case of the diazido $\beta$-CD (Figure 5.1.9 b) the thermogram is same, except that the second mass loss is at a lower temperature of 250°C due to the modification of cyclodextrin. The thermogram of pristine $[60]$fullerene (Figure 5.1.9 c) shows the mass loss after 500°C. The thermogram of $\beta$-cyclodextrin-$[60]$fullerene adduct (Figure 5.1.9 d) showed that the mass loss of the $\beta$-cyclodextrin at 327°C was 75%. This result indicates that one $[60]$fullerene molecule was sandwiched between two cyclodextrins supporting the structure of adduct as shown in Scheme 5.1.1.
Figure 5.1.9 TGA curves of (a) β-CD, (b) diazido β-CD, (c) C\textsubscript{60} and (d) β-CD-C\textsubscript{60} adduct.

5.1.3.5 X-ray Diffraction (XRD)

XRD is widely used technique for the study of supramolecular aggregates to depict the aggregate structure. The X-ray diffraction pattern of C\textsubscript{60}, diazido β-CD and β-CD-C\textsubscript{60} adduct is shown in Figure 5.1.10. The X-ray diffractogram pattern of the formed β-CD-[60]fullerene adduct (Figure 5.1.10 c) showed that the adduct has neither the typical 2θ values of functionalized β-CD (Figure 5.1.10 b) nor those of [60]fullerene (Figure 5.1.10 a). It can be seen that the β-CD-C\textsubscript{60} adduct has a different structure to the functionalized β-CD (2θ = 10.7, 12.5, 19.5, 22.7, 27.0, 34.7°) and [60]fullerene (2θ = 11.0, 17.5, 21.7°), with total suppression of the crystalline structure of β-CD. This type of phenomenon also observed for the β-CD/[60]fullerene inclusion complex.
Figure 5.1.10 X-ray diffractogram of (a) C$_{60}$, (b) diazido β-CD and (c) β-CD-C$_{60}$ adduct.

5.1.3.6 Transmission Electron Micrograph (TEM) and Field Emission Scanning Electron Microscopy (FE-SEM)

The most direct and visible evidence for the supramolecular self-assembly and formation of linear β-CD-[60]fullerene adduct is given by TEM and FE-SEM as shown in Figure 5.1.11. The TEM image (Figure 5.1.11 a) of β-CD-[60]fullerene adduct provides the confirmation for the presence of linear supramolecular nanostructure of 2000-2500 nm, which are joint together linearly through approximately 1000-1200 units of the β-CDs and [60]fullerene molecules. The same nanostructure of β-CD-[60]fullerene adduct also supports from the FE-SEM image (Figure 5.1.11 b). On the basis of TEM and FE-SEM results we suggest, here in a possible structural model of supramolecular self-assembly as shown in Figure 5.1.11 c. The model shows that β-CD-C$_{60}$ adduct is arranged in a channel through a head-to-tail arrangement and forms micrometer size supramolecular self-assembly. Similarly, 2:1 inclusion complex of β-CD and [60]fullerene form unique supramolecular self-assembly was confirmed by TEM as shown in Figure 5.1.12. The size of the self-assembly was found to be
approximately 1000 nm. These self-assemblies were observed due to the strong non-covalent integrations such as van der Waals forces, hydrogen-bonding, hydrophilic/hydrophobic interactions, \( \pi-\pi \) stacking interaction, electrostatic interactions, donor and acceptor interactions, etc.

*Figure 5.1.11* (a) TEM image (b) FE-SEM image and (c) Schematic illustration of the proposed supramolecular self-assembly of \( \beta \)-CD-C\textsubscript{60} adduct.
Figure 5.1.12 TEM image of self-assembly of 2:1 inclusion complex of β-CD and [60]fullerene.
5.1.3.7 Atomic Force Microscopy (AFM)

The freeze-dried β-CD-[60]fullerene adduct was redispersed in distilled water then drops of solution spread over a sheet of mica and dried at room temperature. The picture of a 1 μm² surface of β-CD-[60]fullerene adduct is depicted in Figure 5.1.13. It can be clearly seen that the solid sample contains rather uniformly stacked nanostructure of 200 nm width, which appear to be built up from the conjugation and supramolecular self-assembly of functionalized β-CD and [60]fullerene in a linear manner, which was also supported from TEM and FE-SEM results.

Figure 5.1.13 AFM images of β-CD-[60]fullerene adduct.
5.1.4 Conclusions

Diazido functionalized β-CD synthesized in Chapter 2 was used as reactant and it is easily reacts with the double bond of the [60]fullerene via a [2+3] 1,3-dipolar nucleophilic cycloaddition reaction. Thus, the covalent linking the highly water-insoluble [60]fullerene to a highly water-soluble cyclodextrin derivative gives the water-soluble adduct. The formation of covalent bond and non-covalent inclusion complex in aqueous media between [60]fullerene and diazido modified cyclodextrin has been confirmed from absorbance studies, and the stability of the complex (head-tail structure) has been confirmed from extraction experiment and spectroscopic studies. The supramolecular self-assembly of adduct has been further confirmed from the microscopic (TEM, FE-SEM and AFM) results, which suggest the linear rod like nanostructure of adduct. The tethering of the [60]fullerene molecule to the water-soluble β-cyclodextrins by both covalent linkage and non-covalent interaction has potential biomedical applications, where [60]fullerene properties can be used in aqueous medium.
5.2 Solubilization of [60]Fullerene and [70]Fullerene by Disaccharides

5.2.1 Introduction

Since the discovery of the novel biomedical applications of [60]fullerene attempts have been made to make it water-soluble\textsuperscript{22-31}. Various techniques have been applied to make it water-soluble that include covalent functionalization\textsuperscript{32-34}, emulsion formation\textsuperscript{35,36}, complexation with biomaterials\textsuperscript{37,38} and non-covalent guest-host systems\textsuperscript{39-41}. Among the guest-host systems, cyclodextrins are widely studied and successfully utilized including several patents\textsuperscript{42,43}. One of the fundamental logic of guest-host system is that the fullerene molecule occupies the hydrophobic cavity of the cyclodextrins. Based on this premise it was argued that only γ-cyclodextrin (cavity size = 0.950 nm) could include the [60]fullerene (diameter = 0.7 nm) thus forming a 1:1 inclusion complex which was subsequently modified to show that even β-cyclodextrin (cavity size = 0.78 nm) could include a [60]fullerene molecule forming a 1:2 inclusion complex\textsuperscript{44,45}. This was possible due to the formation of a self-assembled structure which included the [60]fullerene in the cavity formed by two cyclodextrin units\textsuperscript{46,47}. If one were to look at the cyclodextrin structure closely, the glucose units are linked in by the α-1,4-glycosidic linkages\textsuperscript{48}. How important is it for the [60]fullerene molecule to sit inside a cavity made by these bucket structures? If two β-cyclodextrins could include the [60]fullerene molecule then by the same logic it should be possible for a disaccharide to encircle a [60]fullerene molecule due to the same hydrophobic interactions. In fact some early studies have shown that fullerene/carbohydrates compositions were possible by mechanochemical method that led to fullerene aggregates in carbohydrate shell and that surface carbohydrate layer formed a van der Waals complex with fullerene\textsuperscript{49,50}. Subsequent molecular modelling studies and theoretical calculations have shown that the enthalpy of formation of fullerene/sucrose complex was 3.5 kcal/mole\textsuperscript{51}. If that is the case a few number of disaccharides could be used to isolate [60]fullerene in aqueous media or in other words solubilise [60]fullerene.

This chapter describes the preparation of crystalline highly water-soluble [60]fullerene/[70]fullerene by complexation with very inexpensive naturally and commercially available, nontoxic disaccharides (lactose, maltose and sucrose) in comparison with very expensive and difficult to synthesize and toxic host molecules.
Disaccharides can easily interact with [60]fullerene/[70]fullerene and convert this highly water-insoluble molecules into the highly water-soluble complex. Enthalpy and entropy calculation of interaction between [60]fullerene and disaccharides also support the formation of non-covalent complex. The non-covalent interaction between [60]fullerene and disaccharides were characterized by using conventional characterization techniques like UV-Vis, FTIR, NMR, XRD, TGA analysis. The morphology and particles size of the complex was determined by Transmission Electron Micrograph (TEM) and Static Light Scattering (SLS).
5.2.2 Experimental

5.2.2.1 Materials

[60] Fullerene (purity 99.9%), [70] fullerene (purity 99%) and the most stable free radical 2,2'-diphenyl-1-picryl hydrazyl (DPPH) (stored in dry ice) were purchased from Sigma-Aldrich and used as received. Maltose monohydrate, lactose monohydrate, sucrose and spectrophotometric grade toluene, DMSO was purchased from Merck, Mumbai, India. Double distilled water was used during the experimental work.

5.2.2.2 Measurements

The UV-Visible spectra of the solutions were recorded on a Shimadzu-2450, spectrophotometer. The FTIR spectra of the freeze-dried compounds was recorded on a Shimadzu 8400S, Fourier Transform Infrared Spectrometer by using KBr pellet method at 30 scans and $10^{-4}$ resolution at room temperature. XRD patterns were measured on a Rikagu diffractometer with CuKα radiation ($\lambda = 0.15406$ nm) at 40 kV and 40 mA. $^1$H and $^{13}$C NMR spectra were recorded on a JEOL JNM-LA300WB; 400 MHz instrument. All spectra were measured in D$_2$O as solvent and the chemical shifts were referenced to tetramethylsilane (TMS) at 0 ppm. Thermogravimetric analysis (TGA) was recorded on a Shimadzu, TGA-50 system with a heating rate of 10°C/min under air atmosphere in the temperature range of 30-700°C. To study the morphology and particle size of complex, an energy-filtering transmission electron microscopy (TEM) [EF-TEM, EM 912 OMEGA (ZEISS, S-4700), 120 kV] was used. For TEM observation, the 1% complex solutions in double distilled water was drop casted on 400 mesh carbon-coated copper grids and annealed at 100°C for 12 hours before measurement. Particle size was also measured by static light scattering technique with Ga-As semiconductor laser (mini Dawn Tristar, Wyatt). For the molecular modeling studies, the software ChemOffice 2004 (Chem 3D Ultra 8.0 version) was used.
5.2.2.3 Synthesis of the [60]fullerene-disaccharide complex

The complex between [60]fullerene and disaccharides were prepared by using mixed homogeneous solvent system to bring water-insoluble [60]fullerene and water-soluble disaccharide into one homogeneous phase. Thus, disaccharide (lactose monohydrated) 1000 mg (0.28 mmol) was dissolved in polar solvent DMSO and 10 mg [60]fullerene (0.014 mmol) in non-polar solvent toluene, these two solutions were mixed together and stirred for 36 hrs in nitrogen atmosphere at room temperature, during which the deep purple homogeneous solution turned deep brown. The progress of the reaction was determined by taking an aliquot of the reaction mixture after every 4hrs and absorbance measured. After the completion of the reaction the highly water-soluble complex was isolated by removing solvent by vacuum on a rotary evaporator and dissolving the brown solid into excess of water. This aqueous solution was stirred for 30 min and insoluble uncomplexed material was removed by filtration through 0.45 μm syringe-filter. The filtrate aqueous solution of the complex was purified by ultrafiltration over a polymer membrane (MWCO = 1K) to remove excess of disaccharide. The resulting purified concentrated aqueous solution was subjected to freeze-drying to get brown color highly water-soluble [60]fullerene-disaccharide complex. The reaction between lactose and C_{60} is shown in Scheme 5.2.1.

![Scheme 5.2.1 Complexation of lactose-C_{60} in mixed solvent system.](image)

Scheme 5.2.1 Complexation of lactose-C_{60} in mixed solvent system.
5.2.2.4 Determination of Aqueous Solubility of Complex and Quantification of [60]Fullerene

The aqueous solubility of the prepared complex with different disaccharides was determined in terms of weight by dissolving 50 mg of complex sample in the minimum amount of distilled water with the help of μL pipette; ensure the solution reaching saturation at room temperature (30°C). The solubility measured in triplicate and average value was reported.

Concentration of [60]fullerene per mg of the complex was determined by extracting [60]fullerene with toluene from the solid [60]fullerene-disaccharide complex and evaluated from the [60]fullerene standard absorbance in toluene.

5.2.2.5 Radical scavenging

A solution of lactose-C$_{60}$ complex was prepared in water (1%, w/v), and its UV-Vis absorbance spectrum was recorded. The solution of the free radical DPPH was prepared in ethanol (1.7 x 10$^{-4}$ M), and its UV-Vis absorbance spectrum also recorded. Both solutions were kept in a temperature bath at 20°C. In a 20-mL reaction vial, 5 mL of each of the above solutions were mixed, and the UV-Vis absorbance spectrum of the solution mixture was recorded. The reaction temperature was maintained at 20°C, and the contents were constantly stirred. At regular intervals the absorption spectra of the solution mixture were recorded. The reaction of lactose-C$_{60}$ complex and 2,2’-diphenyl-1-picryl hydrazyl (DPPH) is shown in Scheme 5.2.2. Similarly, radical scavenging of DPPH with other prepared water-soluble fullerenes has been also carried out.
Scheme 5.2.2 Reaction scheme for radical scavenging of most-stable free radical DPPH with lactose-C\textsubscript{60} complex.
5.2.3 Results and Discussion

[60] Fullerene is an extremely water-insoluble molecule. Solubilizing the
[60] fullerene in polar solvents is difficult and its solubility in non-polar organic
solvents is also limited. The lack of genuine water-solubility and availability of
macroquantities of [60] fullerene and disaccharides inspired us to prepare water-
soluble [60] fullerene-disaccharides complex for the requirement of biological
applications. Highly water-soluble [60] fullerene-disaccharides complex was prepared
by simple complexation of reactants in homogeneous solvent system. Similarly
disaccharides also solublize [70] fullerene. Among the commercially available
disaccharides, the complex of [60] fullerene with lactose was fully characterized.

To see the visible evidence for the stability of the lactose-C$_{60}$ complex in polar
and non-polar solvents, the extraction experiment was conducted in vials.
[60] Fullerene in toluene shows the deep purple color (Figure 5.2.1 [A]) and it is
completely insoluble in polar solvents. Lactose is completely soluble in polar DMSO
(Figure 5.2.1 [B]). These two solutions when mixed together in inert atmosphere and
after evaporation of organic solvents resulted in a water-soluble slightly yellowish
brown color lactose-C$_{60}$ complex (Figure 5.2.1 [C]). When toluene was added to the
solid lactose-C$_{60}$ complex (Figure 5.2.1 [D]), it was observed that [60] fullerene easily
go into the toluene layer and lactose was settled down at the bottom of the vial. When
toluene was added to the aqueous solution of lactose-C$_{60}$ complex (Figure 5.2.1 [E]),
it was observed that aqueous solution of lactose-C$_{60}$ complex was stable enough and
[60] fullerene does not go into the toluene layer. Thus, the aqueous solution of lactose-
C$_{60}$ complex was stable enough and [60] fullerene was not extractable. Whereas, this is
completely inverted in the case of 2:1 inclusion complex of β-CD with [60] fullerene
as shown in earlier section.

Similar observations made for the lactose-C$_{70}$ complex and are shown in
Figure 5.2.2. [70] Fullerene in toluene shows the reddish wine color (Figure 5.2.2 [A])
and it is completely insoluble in polar solvents. Lactose is completely soluble in polar
DMSO (Figure 5.2.2 [B]). These two solutions when mixed together in inert
atmosphere and after evaporation of organic solvents resulted in a water-soluble
slightly reddish brown color lactose-C$_{60}$ complex (Figure 5.2.2 [C]). When toluene
was added to the solid lactose-C$_{70}$ complex (Figure 5.2.2 [D]), it was observed that
[70]fullerene easily go into the toluene layer and lactose settled down at the bottom of the vial. When toluene was added to the aqueous solution of lactose-C\textsubscript{70} complex (Figure 5.2.2 [E]), it was observed that aqueous solution of lactose-C\textsubscript{70} complex was stable enough and [60]fullerene does not go into the toluene layer. Thus, the aqueous solution of lactose-C\textsubscript{70} complex was stable enough and [70]fullerene was not extractable.

![Figure 5.2.1 Photograph of [A] C\textsubscript{60} in toluene, [B] lactose in DMSO, [C] lactose-C\textsubscript{60} complex in water, [D] lactose-C\textsubscript{60} complex in toluene and [E] lactose-C\textsubscript{60} complex in water + toluene.](image)

**Figure 5.2.1** Photograph of [A] C\textsubscript{60} in toluene, [B] lactose in DMSO, [C] lactose-C\textsubscript{60} complex in water, [D] lactose-C\textsubscript{60} complex in toluene and [E] lactose-C\textsubscript{60} complex in water + toluene.

![Figure 5.2.2 Photograph of [A] C\textsubscript{70} in toluene, [B] lactose in DMSO, [C] lactose-C\textsubscript{70} complex in water, [D] lactose-C\textsubscript{70} complex in toluene and [E] lactose-C\textsubscript{70} complex in water + toluene.](image)

**Figure 5.2.2** Photograph of [A] C\textsubscript{70} in toluene, [B] lactose in DMSO, [C] lactose-C\textsubscript{70} complex in water, [D] lactose-C\textsubscript{70} complex in toluene and [E] lactose-C\textsubscript{70} complex in water + toluene.
5.2.3.1 Aqueous Solubility of Complexes

As expected, after complexation of [60]fullerene with different disaccharides, the aqueous solubility of [60]fullerene increased significantly as shown in Table 5.2.1. The complex of maltose with [60]fullerene has high water-solubility due to the maltose has highest water-solubility than other disaccharides. The complex of maltose-C$_{60}$ has more [60]fullerene concentration due to the higher solubilizing power of maltose. Moreover, it was found that aqueous solution of the complex was stable for the several months and does not aggregate like β-cyclodextrin-[60]fullerene complex. The amount of [60]fullerene/mg of the complex was determined by UV-Vis after extraction with toluene. It was observed that the amount of [60]fullerene in complex was found to be less than 10%.

Table 5.2.1 Aqueous solubility of corresponding complex at 25°C and concentration of C$_{60}$ per mg of complex

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solubility (mg/mL)</th>
<th>Solubility of Corresponding Complex (mg/mL)</th>
<th>Concentration of C$_{60}$/mg of Complex (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>160.0</td>
<td>55.5</td>
<td>77.40</td>
</tr>
<tr>
<td>Maltose</td>
<td>1080.0</td>
<td>71.4</td>
<td>90.02</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.0</td>
<td>42.3</td>
<td>51.20</td>
</tr>
<tr>
<td>β-cyclodextrin</td>
<td>18.5</td>
<td>4.0</td>
<td>77.30</td>
</tr>
</tbody>
</table>

5.2.3.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is an effective tool to characterize the non-covalent interactions between molecules. The FTIR absorption spectra of C$_{60}$, lactose and C$_{60}$-lactose are shown in Figure 5.2.3. The spectrum of pristine C$_{60}$ (Figure 5.2.3 a) showed four characteristic absorption bands at 1429 cm$^{-1}$, 1183 cm$^{-1}$, 578 cm$^{-1}$ and 527 cm$^{-1}$. The spectrum of lactose (Figure 5.2.3 b) showed the absorption band at 3335 cm$^{-1}$, 1653 cm$^{-1}$, 1038 cm$^{-1}$ and 779 cm$^{-1}$. The spectrum of C$_{60}$-lactose complex
(Figure 5.2.3 c) showed 3334 cm\(^{-1}\), 1653 cm\(^{-1}\), 1035 cm\(^{-1}\) and 777 cm\(^{-1}\) of lactose and also showed distinct IR absorption bands of C\(_{60}\) at 578 cm\(^{-1}\) and 527 cm\(^{-1}\). The lactose peaks remains strong in the spectrum, the positions and relative intensities of a few bands are affected by the formation of non-covalent complex between lactose and C\(_{60}\). These results indicate the modification of environment of lactose due to the formation of lactose/C\(_{60}\) complex. If it were not so then the spectra would resemble that of a physical mixture of C\(_{60}\) and the lactose with no shift in the characteristic bands.

![FTIR absorption spectra](image)

**Figure 5.2.3** FTIR absorption spectra of (a) C\(_{60}\), (b) lactose and (c) lactose-C\(_{60}\) complex.

### 5.2.3.3 UV-Visible Spectroscopy (UV-Vis)

The UV-Vis absorbance spectra of lactose in water, C\(_{60}\) in toluene and lactose-C\(_{60}\) complex in water is shown in Figure 5.2.4. The UV-Vis absorbance spectrum of C\(_{60}\) in toluene shows maximum absorbance at 334 nm which is completely insoluble
in water, whereas lactose in water does not show any absorbance in this region. However, the UV-Vis absorbance spectrum of lactose-C_{60} complex in water showed a maximum absorbance at 348 nm and widening of absorbance band after 400 nm as compared to typical absorbance at 334 nm and 406 nm for the pristine fullerene in toluene. Another interesting observation was that fullerene could be extracted from the dry powder of the complex by shaking it with toluene. The UV-Vis spectrum (Figure 5.2.4, Inset) showed absorbance similar to that of pristine [60]fullerene in toluene, whereas this was not possible in the aqueous solution, unlike the β-cyclodextrin-[60]fullerene complex (toluene can penetrate into the nonpolar cavity of β-cyclodextrin and kick out the [60]fullerene from the cavity), thus giving an indication that toluene is unable to penetrate into the complex as toluene does not dissolve the disaccharides\textsuperscript{52}.  

![Graph showing UV-Vis spectra of lactose, C_{60}, lactose-C_{60} complex, and inset C_{60} extracted from complex by toluene.]

**Figure 5.2.4** UV-Vis spectra of lactose, C_{60}, lactose-C_{60} complex and inset C_{60} extracted from complex by toluene.
5.2.3.4 Thermogravimetric Analysis (TGA)

The stoichiometry of the lactose-C\textsubscript{60} complex was evaluated from the thermogravimetric analysis as shown in Figure. 5.2.5. A small mass loss due to the loss of structural water molecules at the beginning was found in the lactose and lactose-C\textsubscript{60} complex followed by a larger mass loss corresponding to the decomposition of the glucose units of the lactose. The second mass loss for the lactose-C\textsubscript{60} complex was observed to be at lower temperature compared to pristine lactose, due to the modification of glucose units environment after formation of complex with [60]fullerene. Generally, lactose can form complexes with [60]fullerene in the ratio of (1:1, 2:1, 3:1, 4:1,...) with possible ratio of (1:2, 2:2, 3:2, 4:2,...). The theoretical, calculated mass content of lactose in the complex for these ratio could be (32.20\%, 48.71\%, 58.76\%, 65.52\%,...) and also possibility of (19.19\%, 32. 20\%, 41.60\%, 48.57\%,...). The TG data of the lactose-C\textsubscript{60} complex show that the total mass loss at 375°C is 65.18\%\textsuperscript{34-55}. This value indicates that the ratio between lactose and C\textsubscript{60} complex is 4:1.

![Figure 5.2.5 TGA curves of lactose, C\textsubscript{60} and lactose-C\textsubscript{60} complex.](image)
5.2.3.5 Nuclear Magnetic Resonance Spectroscopy (NMR)

Further corroboration for the formation of non-covalent complex was obtained through NMR spectroscopy. Proton nuclear magnetic resonance provided direct evidence of supramolecular non-covalent interactions and this was confirmed by upfield or downfield shifts of the proton signals\textsuperscript{34,35}. The $^1$H NMR spectra (Figure 5.2.6) of the complex showed a considerable upfield shift of the parent protons of lactose due the formation of complex and $^{13}$C NMR spectra (Figure 5.2.7) of the lactose-C$_{60}$ complex shows all the carbon chemical shift of the parent lactose along with the chemical shift at 165.27 ppm which is due to the presence of C$_{60}$ with some chemical shift of the lactose carbon skeleton\textsuperscript{36}.

![NMR Spectra](image)

**Figure 5.2.6** $^1$H NMR spectra of lactose and lactose-C$_{60}$ complex in D$_2$O.
**Figure 5.2.7** $^{13}$C NMR spectra of lactose and lactose-C$_{60}$ complex in D$_2$O.

### 5.2.3.6 X-ray Diffraction (XRD)

All the complexes were subjected to XRD investigation and surprising result was that the complexes were highly crystalline. Figure 5.2.8 depicts the X-ray diffraction pattern of the C$_{60}$, lactose and freeze-dried lactose-C$_{60}$ complex. The signals were observed at (2θ=12.44, 16.32, 19.10, 19.52, 19.92, 20.78, 21.16, 22.72, 23.66, 25.52, 27.40, 36.20, 37.88°) and the product isolated has nearly similar peaks corresponding to lactose with some decrease in the intensity and little peak shift. It can be seen that the complex has a similar crystalline structure to the parent lactose (2θ=12.36, 16.12, 18.82, 19.28, 19.7, 20.56, 21.02, 22.54, 23.56, 25.34, 27.24, 35.88, 37.28°) and [60]fullerene (2θ=10.68, 17.58, 20.68, 21.60, 27.34, 28.04, 30.74, 32.70°), with the retention of the crystalline structure of lactose$^{52,53}$. This type of behaviour is completely different from the inclusion complex of [60]fullerene with any crystalline host where the host looses its crystalline nature and becomes amorphous$^{55,58}$. Thus, it was obvious that the crystalline complexes were formed due to the self-assembly of the disaccharide and the fullerene molecules led to arrangement of molecules in directional order.
Figure 5.2.8 X-ray diffractogram of (a) C\textsubscript{60}, (b) lactose and (c) lactose-C\textsubscript{60} complex.

5.2.3.7 Transmission Electron Micrograph (TEM) and Static Light Scattering (SLS)

The unique self-assembly and retention of the crystalline nature of the lactose-C\textsubscript{60} complex was further confirmed by the transmission electron micrographs (TEM) as shown in Figure 5.2.9 (a). The lactose-C\textsubscript{60} complex was found spherical in shape with uniform crystalline aggregates of about 60±5 nm. The particles size obtained from static light scattering (SLS) measurements as shown in Figure 5.2.10 also support the prior inference drawn from the TEM micrographs. Even though the concentration of complex in aqueous solution was increased, the particle size does not change and was found to be 60±5 nm. High resolution TEM (HRTEM) shows a regular array of the [60]fullerenes with lactose with the lattice spacing between the fullerene molecules of the order of 0.34 nm (Figure 5.2.9 (a), Inset). This is similar to the lattice spacing between the [60]fullerene crystal planes in the solid state\textsuperscript{59,61}. Moreover, the lactose-C\textsubscript{60} complex shows self-assembly as shown in Figure 5.2.9 (b) due the strong non-covalent integrations such as van der Waals forces, hydrogen-
bonding, hydrophilic/hydrophobic interactions, π-π stacking interaction, electrostatic interactions, donor and acceptor interactions, etc.

**Figure 5.2.9** (a) TEM micrograph of lactose-C₆₀ complex; inset: HRTEM image of C₆₀ lattice (b) spherical self-assembly of lactose-C₆₀ complex (c) histogram of lattice spacing.

**Figure 5.2.10** Particle size of lactose-C₆₀ complex by static light scattering.
5.2.3.8 Molecular Modeling

Different views of space-filling molecular models of the three-dimensional conformation of the lactose-C\textsubscript{60} complex are shown in Figure 5.2.11. The host and guest molecules were independently built up, and their geometry was optimized. The overall structure of these possible models of complex was again subjected to energy-minimization. The final energy-minimized molecular model indicates that C\textsubscript{60} was surrounded by four lactose molecules in a spherical form. This model also supported from the results obtained by TEM analysis as shown in Figure 5.2.9, lactose molecules are arranged spherically around the [60]fullerene. Furthermore, such units are coming together to form self-assembly about 60 nm and then these self-assembly coming together to form 500 nm cluster.

![Space-filling energy-minimized (MM2) molecular models showing different views of lactose-C\textsubscript{60} complex.](image)

**Figure 5.2.11** Space-filling energy-minimized (MM2) molecular models showing different views of lactose-C\textsubscript{60} complex.

5.2.3.9 Radical Scavenging

The stable free radical has been used to study the antioxidant activities of phenols and catechol. In these reactions, the antioxidant transfers a hydrogen atom to the radical, which leads to a decrease of the characteristic UV-Vis absorption intensity in the spectrum of DPPH. To demonstrate the radical scavenging property of water-soluble [60]fullerene we utilized the DPPH reaction with lactose-C\textsubscript{60} complex. The progress of the radical scavenging reaction of lactose-C\textsubscript{60} complex was measured by taking UV-Vis absorbance at definite interval of time as shown Figure 5.2.12. It was observed that pristine DPPH in ethanol shows maximum UV-Vis absorbance at 517
nm, while lactose-C₆₀ complex in water shows maximum UV-Vis absorbance at 348 nm. When these two solutions were mixed together the maximum UV-Vis absorbance of DPPH shifted to 527 nm from 517 nm. It could be further seen that the absorbance of this maximum decreased steadily with time and we can observe that the pink colour of the radical being bleached gradually. The reaction of DPPH with water-soluble lactose-C₆₀ complex proceeded at a comparatively slower rate, the pink color of the radical being bleached gradually. Nevertheless, the decrease in absorbance is detectable within minutes of the reaction. To observe the solvent effect on DPPH scavenging blank experiment was conducted without lactose-C₆₀ complex by mixing DPPH solution and water. However, there was no considerable change in absorbance at the characteristic peak maximum was observed even after several hours of contact.

![Absorbance vs Wavelength](image)

**Figure 5.2.12** Color bleaching UV-Vis spectra of radical scavenging of most stable free radical DPPH with lactose-C₆₀ complex.

**5.2.3.10 Kinetics of Radical Scavenging Reaction**

The lactose-C₆₀ complex has a large number of reaction sites on its π-surface of C₆₀; each C₆₀ molecule is capable of reacting with a number of DPPH radicals.
Hence, in terms of molar equivalents, the concentration of C$_{60}$ in effect is very high compared to that of DPPH during the initial stages of the reaction, while the concentration of DPPH would decrease steadily with time, the concentration of lactose-C$_{60}$ complex would practically remain constant. The reaction between lactose-C$_{60}$ complex and DPPH follows the first-order reaction rate law $\ln [a(a - x)^{-1}] = kt$. The plot of the term $\ln [a(a - x)^{-1}]$ vs. reaction time ($t$, min) is shown in Figure 5.2.13, where $a$ denotes the initial DPPH concentration and $(a - x)$ the concentration of DPPH at different reaction times, evaluated from the DPPH standard curve, led to a straight line with a satisfactory correlation of 0.9814. The pseudo-first-order rate constant ($k$) was evaluated to be $1.05 \times 10^{-3}$ min$^{-1}$. However, there was a deviation from linearity beyond 120 min, indicating that the pseudo-first-order reaction condition no longer remains valid beyond this stage i.e., the concentration of lactose-C$_{60}$ complex begins to perceptively decrease beyond a certain stage of the reaction. The pseudo-first-order reaction of DPPH observed in the early stages of the reaction further indicates that multiple sites of each C$_{60}$ molecule reacted with DPPH.

![Graph showing the relationship between $\ln [a(a - x)^{-1}]$ and time (min).]

**Figure 5.2.13** Pseudo-first-order rate correlations for DPPH in the reaction between DPPH and lactose-C$_{60}$ complex.
5.2.4 Conclusions

The present study demonstrated an eco-friendly and low cost protocol for the aqueous solubilization of fullerenes by self-assembly with disaccharides. The identification of lactose complex with [60]fullerene suggested that one [60]fullerene ball was surrounded by four lactose molecule in a spherical nanostructure with retention of crystalline nature of the parent compounds. The self-assembly was formed due to the van der Waals force, charge transfer complex and hydrogen bonding. The solubility and stability of [60]fullerene-disaccharide complex was found to be higher than the traditional [60]fullerene-cyclodextrins inclusion complex. The similar observation has been observed for the [70]fullerene-disaccharides. The preliminary radical scavenging studies with most stable free radical DPPH in aqueous system suggest that the complex has potential bio-medical application.
References


42. C. N. Murthy and K. E. Geckeler, *Korean patent no*, 10-0479331, **2005**.

43. C. N. Murthy and K. E. Geckeler, *Korean patent no*, 10-20020047438, **2004**.


