Chapter 2

EXPERIMENTAL

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2.1 HOST-GUEST COMPLEXES OF [60]FULLERENE

The host-guest complexes of [60]fullerene have been extensively studied since its discovery. The basic motive was to encapsulate an essentially nonpolar molecule to make it water-soluble. The other reason for the encapsulation was for the purpose of selective separation of the class of fullerenes based of the size differences between them. Thus macrocyclic entities like cyclodextrins, calixarenes, porphyrine and tetrathiofulvalenes among others have been reported to encapsulate fullerenes. The driving force for the formation of the host-guest complexes is the non-covalent interactions between the two. Among the less studied host has been lysozyme that has an apolar cavity and is water-soluble thus making it a potential candidate for the formation of a host-guest complex with [60]fullerene.

2.1.1 LYSOZYME AS A BIOMOLECULE AND ITS INTERACTIONS WITH [60]FULLERENE

The biomedical applications of fullerene imply its possible reaction with most essential cellular components like nucleic acids and proteins. Earlier studies on DNA-[60]fullerene interactions have shown that the [60]fullerene cleaves DNA and is base specific[69]. It has also been shown that [60]fullerene interaction with HIV virus protease inhibited the activity of the latter[70]. The specific role of the [60]fullerene in these systems is not very clear. To gain further insight, protein-fullerene interactions were studied using lysozyme as a model protein.

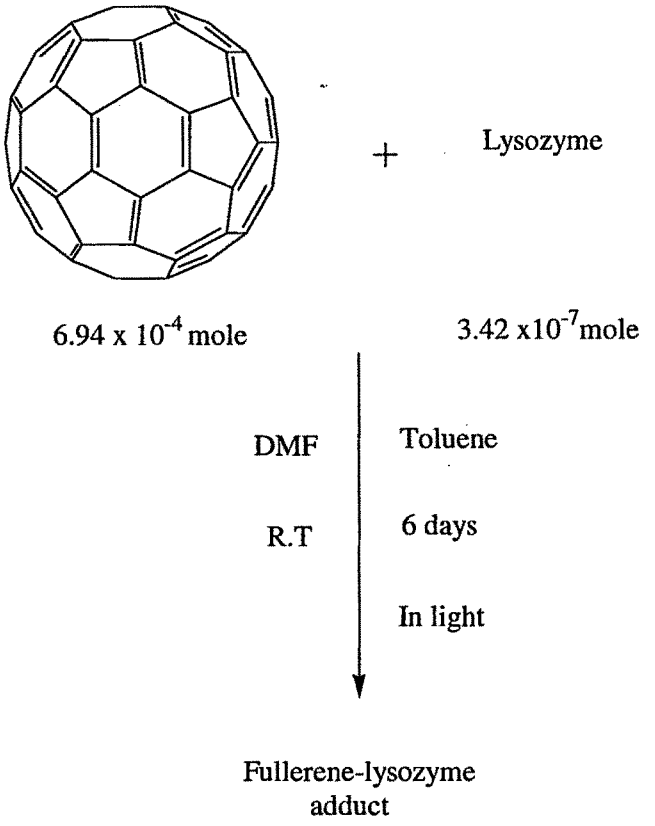
2.1.1.1 Synthesis of [60]fullerene-lysozyme adduct

Materials

[60]Fullerene and lysozyme were purchased from M/s E. Merck, Germany. Tóluene, dimethylformamide (DMF) were purchased from M/s Merck(I) Ltd., and dried using standard procedures before use.

Method

In a 30ml glass vial 5mg of lysozyme dissolved in 5ml of DMF-water solution was taken. To this solution 0.5mg of [60]fullerene dissolved in 0.5ml of toluene was added. The contents were kept for stirring for 6 days reaction at room temperature in the presence of light. After the complete reaction, toluene was removed from the reaction mixture using rotary evaporator under vacuum. The resulting solution was poured into 20ml of water and freeze-dried to get an off white solid product. The same process was repeated in the absence of light and reaction was monitored for 6 days. The typical reaction is shown in Scheme 2.1.



Scheme 2.1. Synthesis of the [60]fullerene-lysozyme adduct

2.1.2 [60]FULLERENE-ITS FORMATION OF REVERSE MICELLE AND ITS MICROEMULSION STUDY WITH VARIOUS NON-IONIC SURFACTANTS.

Incorporation of C_{60} into systems of the micellar or vesicular type has attained a lot of attention. Most of these studies have been limited to the determination or enhancement of the water-solubility the essentially nonpolar and insoluble [60]fullerene. One of the earliest observation of the colloidal formation or aggregation in aqueous media reported a particle size of ≤0.22μm[111]. The incorporation of fullerenes into dioctadecyl dimethylammonium bromide [112] dihexadecyl hydrogen phosphate [112], Triton X-100[112], phosphatidylcholine [113] and poly (vinylpyrrrolidone) has been reported. With the increasing applications of fullerenes in not only biomedical applications but also in the area of photonics and for light harvesting applications, the aggregation study and other colloidal behavior has not been done yet. Thus the microemulsion studies and the formation of [60]fullerene reverse micelles has not been given any attention yet. Thus an attempt has been made to understand the solution behavior of [60]fullerene both in microemulsions and in reverse micelles using different nonionic surfactants. The use of nonionic surfactants as compared to ionic surfactants was to minimize the specific interactions that the [60]fullerene molecule is known to have with different solvents during the solublization process.

2.1.2.1 [60]fullerene in reverse micelle

Materials

Surfactants Span 60 and Brij 35 (Koch light laboratories Ltd, England) were used without any further purification, Toluene (Merck India) was dried under 4Å molecular sieves and was freshly distilled before use. A solution of [60]fullerene in toluene(10 mg per 100ml,1.39 x 10^{-4} M) was made and used as a solvent in this study.

Method

The transmittance of the [60]fullerene solution was determined by spectrophotometer (Elico-SL171) at room temperature and was found to be 85%. A known weight (0.68gm) of Span 60 was taken in the volumetric flask and dissolved in the [60]fullerene solution to make a 3.3 x 10^{-3} M solution of Span 60. Different quantities of the stock solution was taken in separate volumetric flask & and diluted using the 1.39 x 10^{-4} M [60]fullerene solution. Thus all solutions had same concentration of [60]fullerene but different concentration of Span 60. These solutions were kept in a temperature controlled bath and the absorbance measured at 30°C and 40°C at 455nm. The same process was repeated for the structurally different non-ionic surfactant Brij 35 and from the plots of the absorbance and concentration, the critical reverse micelle concentrations were calculated from the break point of the plot for both the surfactants. The probable structure of the reverse micelle is shown in Figure 2.1.

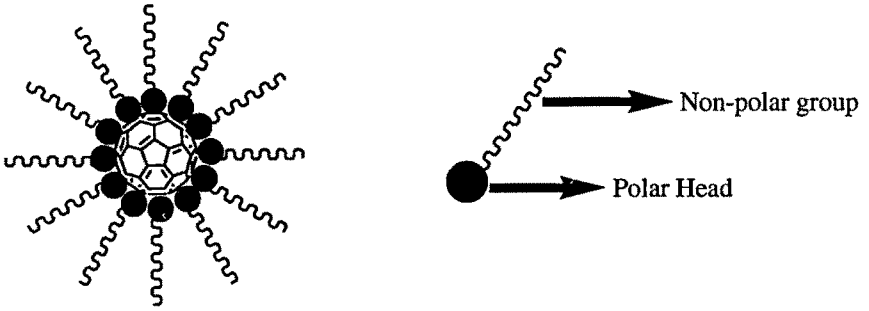


Figure 2.1. Probable structure of the reverse micelle of [60]fullerene with the non-ionic surfactant

2.1.2.2. Microemulsion Studies

Materials

[60]Fullerene, Span 60 (Koch light laboratories Ltd, England), Brij 35 (Merck Schuchardt) and Brij 98 (Sigma-Aldrich ,Germany), toluene(Merck India Ltd), propanol, butanol, pentanol were purchased from Merck India Ltd., and distilled before use. Double distilled water was used through out the microemulsion studies.

Method

The solution of [60]fullerene in toluene formed the oil phase and propanol was the cosurfactant in the microemulsion system. 1.0 ml of the [60]fullerene solution (1.39 x 10^{-4} M) was taken in a test tube and 0.085 gm of Span 60 was added to it and dissolved. To this solution 1.0 ml of propanol was added as a cosurfactant when a homogenous solution was formed. This solution was titrated by sequential addition of 0.1 ml of water. When the water was added a white turbid layer was formed at the interface which disappeared when the contents were slightly shaken. This process was continued till the turbidity was retained even after the contents were shaken. This was taken as the point of transition from microemulsion to emulsion. The procedure was repeated by using different types and quantity of cosurfactant (butanol and pentanol) noting the water required for the transition. Based on these results triangular phase diagrams were plotted for different non-ionic surfactants.

2.1.3. CALIX[8]ARENE AS HOST MOLECULE FOR [60]FULLERENE

Linking the fullerene chemistry with the wide field of the chemistry of the calixarenes is one of the challenges in the chemical research. In 1992 it was reported by Williams and his group that C_{60} can be solubilized in water by using calix[8]arene. Here we have adopted a different approach to make [60]fullerene water soluble by encapsulating it with water-soluble calix[8]arene molecule. First the parent p-tert-butyl calix[8]arene was synthesized by the standard method reported and then it was made water soluble by replacing p-tert-butyl group with sulphonate as well N, N-diallyl amine group on the upper rim of calix[8]arene.

2.1.3.1. Synthesis of p-tert-butyl Calix[8]arene

Materials

p-tert-butylphenol was purchased from Sisco Research laboratory Pvt. Ltd., Mumbai, India, para formaldehyde (Merck India Ltd.), Potassium hydroxide (Merck Ltd), xylene, toluene, diethylether, acetone, chloroform solvents were purchased from Merck India Ltd. and dried through standard procedure before use.

Method

A three necked flask was positioned on a heating mantle. The flask was equipped with a condenser and gas inlet and a magnetic needle was placed inside it. The heating mantle was equipped with magnetic stirrer. This reaction assembly was purged with nitrogen gas for 10 mins and after that 5gm (0.033 mole) p-tert-butyl phenol, 1.75 gm (o.o58 mole) para formaldehyde, 0.1ml of 10 M potassium hydroxide solution and 30 ml of xylene were sequentially added to this flask. The reaction mixture was refluxed for 4 hrs and was allowed to cool to room temperature. Precipitates were collected on a buckner funnel and washed in sequence with 20 ml portion of toluene, diethyl ether, acetone and water respectively. The product was dried and recrystallised using 60 ml of chloroform.

2.1.3.2. Synthesis of p-HCalix[8]arene (dealkylated calix[8]arene)

Materials

The product obtained above was used after purification. Phenol and anhydrous $AlCl_3$ were purchased from Merck India Ltd., and dry toluene was used for the synthesis of p-HCalix[8]arene.

Method

In a three-necked flask 3.0 gms (4.05 mmole) p-tert-butylcalix[8]arene, 45 ml toluene and 1.5 gms (5.58 mmole) phenol was taken & stirred for 10 minutes when a heterogeneous mixture was obtained. Then 5 gms (37.51 mmole) anhydrous $AlCl_3$ was added at once & continued to stir the mixture, which rapidly turned deep red within 30 minute. A viscous deep red sticky phase separated on the walls of the flask which was stirred at room temperature for additional 3 hours. The completion of reaction was conformed by TLC. After completion of the reaction, the reaction mixture was poured into a beaker containing a mixture of ice cold water & 1M HCL. This was transferred into a separating funnel. The funnel was swirled and the organic phase separated. This was then washed with water up to neutralization by observeing the pH of the product using a pH strip. Then the solvent was distilled under reduced pressure using a rotary evaporator (Labrota 4000). The product obtained was titrated with diethyl ether & filtered. An orange solid was obtained which was recrystallized from methanol/chloroform mixture to obtain a light grey solid.

2.1.3.3. Synthesis of p-sulfonato Calix[8]arene

Materials

De alkylated calix[8]arene, concentrated sulfuric acid, barium carbonate and sodium carbonate were purchased from Merck India Ltd., ethanol and distilled water were used for the synthesis of p-sulfonato calix[8]arene.

Method

Calix[8]arene (0.5 gm,0.38 m mole) was mixed with 5ml of concentrated sulfuric acid (0.0948 mole) and the solution was heated at 80°C for 3 hrs. An aliquot was withdrawn from the solution and poured in to water to determine the progress of reaction. The reaction was completed when no water-insoluble material was detected. The precipitate was dissolved in water and the solution neutralized with barium carbonate. Precipitated barium sulphate was removed by filtration and then sodium carbonate was added to the filtrate in order to exchange the counterion. The solution was then concentrated and ethanol was added to the remaining solution to get the sulfonated product which is highly water soluble with light gray colour.

2.1.3.4 Synthesis of water-soluble [p-(N, N-Diallylaminomethyl)] calix[8]arene

Materials

The dealkylated calix[8]arene synthesized and reported previously was used for this reaction. Diallylamine (99%) was purchased from Sigma Aldrich ,Germany. 37% formaldehyde, acetic acid, dimethylsulfoxide (DMSO), potassium carbonate, diethyl ether were purchased from Merck India Ltd and used for this synthesis.

Method

0.5 gm (0.94 mmole) of dealkylated calix[8]arene was dissolved in 20ml of DMSO. To that 10 ml of acetic acid was added. This mixture was stirred for 5 mins. Then 0.92 gms (11.3 mmole) of 37% formaldehyde and 1.4 gms (14.1 mmole) diallylamine were added. The reaction mixture was stirred for 12 hours at room temperature. It was then poured into 100 ml of water to give a clear solution. Upon addition of a solution of K_2CO_3 a precipitate was formed which was separated by filtration, dissolved in diethyl ether and filtered. Evaporation of solvent gave a solid product.

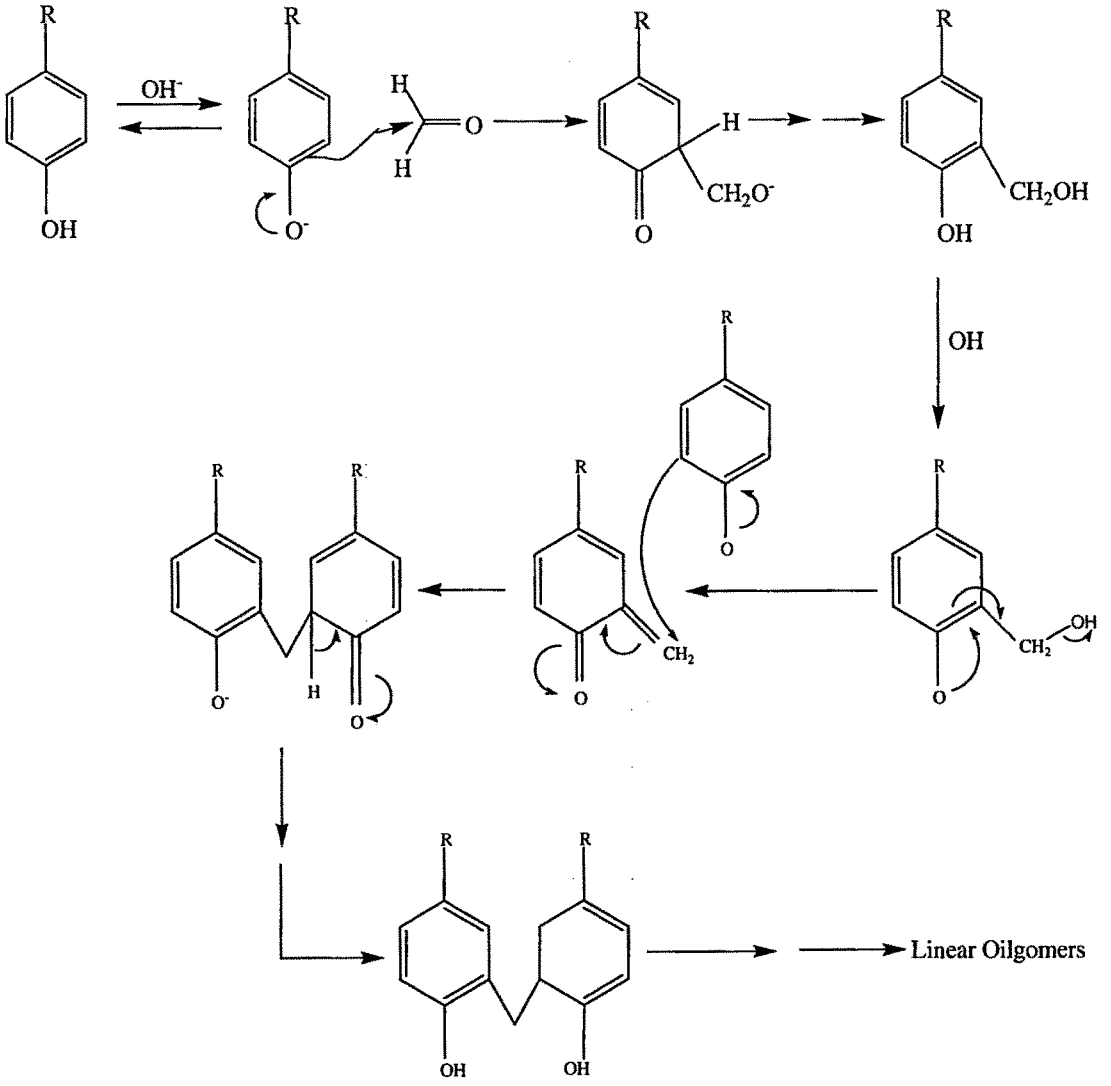
2.1.2.5. Synthesis of water-soluble Calix[8]arene-[60]fullerene complex

Materials

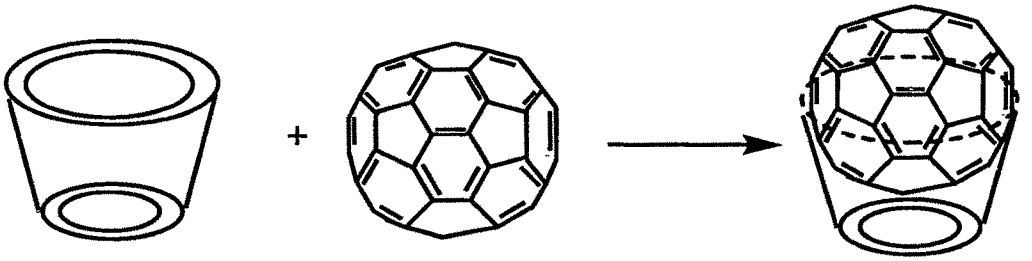
p-SulfonatoCalix[8]arene, [p-(N, N-Diallylaminomethyl)]Calix[8]arene were used as a host molecule. [60]fullerene purchased from M/s E. Merck was used as a guest molecule for this host-guest complexation study, while solvents like toluene and DMF were used which were purchased from the M/s Merck India Pvt. Ltd.

Method

In a 25 ml round bottom flask a 0.5 mg of water soluble calix[8]arene dissolved in 1 ml of DMF was taken to which 1mg of [60]fullerene dissolved in 10ml of toluene was added and reaction mixture stirred on a magnetic stirrer at room temperature. A clear colour change was observed in the reaction flask which indicates the complex formation between the water-soluble calix[8]arene and [60fullerene molecule. After that reaction was stopped and solvent from the reaction mixture was first removed using rotary evaporator. Removal of solvent leaves a brown coloured solid mass to which a 20ml of water was added which is of yellow in colour. This yellow coloured liquid was freeze dried to get the solid mass which was highly soluble in water.



Scheme2.2 Reaction mechanism for the formation of base catalyzed p-tert-butyl calix[8]arene



Scheme 2.3. Synthesis of water-soluble calix[8]arene-[60]fullerene complex

2.1.4 CYCLODEXTRINS AS HOST MOLECULES FOR [60]FULLERENE

The supramolecular chemistry of cyclodextrins (CD) in aqueous solution is a topic of commercial interest with a variety of biomedical applications. Nonpolar drug molecules have been solubilized in water by making their inclusion complexes with cyclodextrins. [60]Fullerene is a non-polar molecule and much of the research on the inclusion complexes of cyclodextrins and [60]fullerene has centered on γ-cyclodextrin[67], considering the cavity size to accommodate a large hydrophobic molecule like C_{60}. In fact there are reports which says that the reason for the unsuccessful attempts to form inclusion complexes of β-cyclodextrin and [60]fullerene is not only the size but it seems to also depends on the reaction conditions[68]. Here this chapter describes the method for the solvent free synthesis of water-soluble γ and β-cyclodextrin-[60]fullerene complex. Further, more the magnetic γ-CD-[60]fullerene complex was also synthesized.

2.1.4.1 Water-soluble solvent free synthesis of γ,β-cyclodextrin-[60]fullerene complex.

Materials

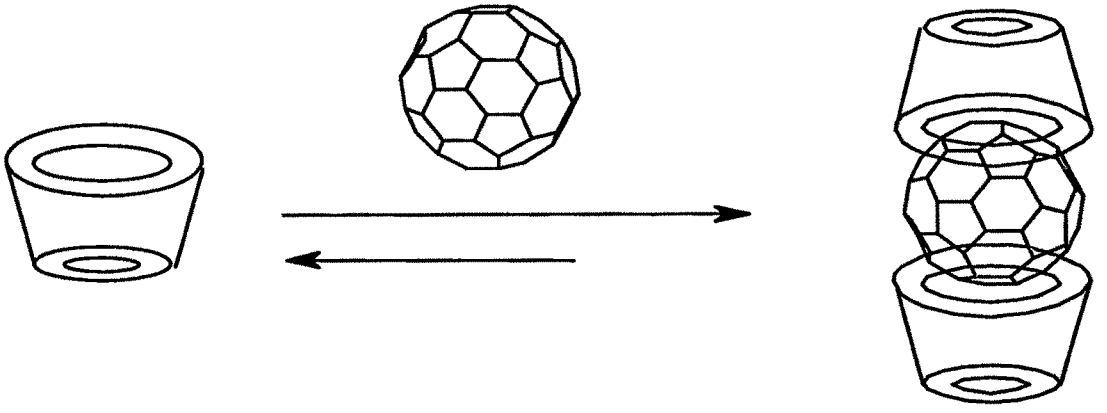
γ-cyclodextrin was purchased from Sigma Aldrich, Germany, β–Cyclodextrin was purchased from Signet chemical corporation, Mumbai, [60]fullerene and distilled water were used for the synthesis of water soluble γ , β-cyclodextrin-[60]fullerene complex.

Method

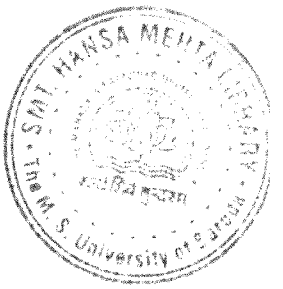
The water-soluble cyclodextrin capped fullerene was prepared by a modified procedure as reported[68]. Equimolar quantities of γ, β -cyclodextrin and C_{60} were ground in an agate mortar for one hour and the mixture was dissolved in distilled water, a yellow solution was formed. This aqueous solution was freeze-dried to get a buff colored product.

2.1.4.2 Photolysis of capped fullerene (γ–CD-C_{60})

A cyclodextrin-fullerene adduct was prepared that was water-soluble and then subject to short pulses of high-energy ultraviolet radiation in air to compensate for the blocking of the fullerene surface to the ultraviolet radiation. This led to the burning of the sample and so a lower energy radiation was used. A definite weight of the adduct was exposed to a low energy (8w) UV radiation for 24 hrs. This sample was taken for magnetic studies on a Quantum Design MPMS2 magnetometer. Both zero field cooled and field cooled measurements were made.



Scheme 2.4. Schematic representation of the encapsulation of [60]fullerene by two γ,.β – cyclodextrin molecules.



2.2. CHARACTERIZATION

2.2.1 *Ultraviolet and visible Spectroscopy*

Ultraviolet and visible spectroscopy (Electronic spectroscopy) is primarily used to measure the multiple bond or aromatic conjugation with in molecules. All the above synthesized water soluble supramolecular [60]fullerene complexes were characterized using a SHIMADZU UV-2450" UV-visible spectrophotometer and the spectra of the water-soluble [60]fullerene-lysozyme adduct is shown in the Figures 3.2(a-e).

The spectra of the [60]fullerene reverse micelle in the Figure 3.5 and Figure 3.7.

The spectra of the water-soluble p-sulfonated calix[8]arene-[60]fullerene complex in are shown in Figure 3.31 and [p-(N,N-diallylaminomethyl)]calix[8]arene-[60]fullerene complex in the Figure 3.33. UV-spectra of γ, β -cyclodextrin-[60]fullerene complex is shown in the Figure 3.40 and Figure 3.41 respectively, of the γ-cyclodextrin-[60]fullerene complex irradiated for 24hrs is in the Figure 3.47.

2.2.2 *Fourier transformer infrared spectroscopy (FTIR)*

This is one of the most widely used tools for the detection of functional groups in pure compounds and mixtures and for compound comparison. The spectrum is obtained in minutes using a few mg of the compound which can also be recovered.

In infrared spectrophotometer, infrared radiation of successively increasing wavelength is passed through the sample of compound and the percent transmittance measured. An infrared spectrum is the graph of per cent transmittance versus either increasing wavelength or decreasing frequency. Each dip in spectrum called band or peak represents absorption of infrared radiation at that frequency by sample.

The Fourier transform infrared spectra of all the synthesized organic compounds were taken on "SHIMADZU FTIR-8400S" Fourier transform infrared spectrophotometer before further synthesis. The spectra were taken on KBr discs of the samples. The FT-IR spectra of the water-soluble [60]fullerene-Lysozyme adduct is shown in the figures 3.3, of p-tert-butyl Calix[8]arene , of p-H calix[8]arene, of p-sulfonato calix[8]arene, of [p-(N, N-Diallylaminomethyl)]calix[8]arene in the figure 3.19, 3.23, 3.25, 3.27 respectively. The FT-IR spectra of water-soluble p-sulfonated calix[8]arene-[60]fullerene complex is shown in the figure 3.30. FTIR Spectra of γ, β -cyclodextrin-[60]fullerene complex is

shown in the Figure3.38 and 3.39 respectively, of the γ-cyclodextrin-[60]fullerene complex irradiated for 24hrs is shown in the Figure 3.46.

2.2.3 *Nuclear magnetic resonance spectroscopy (NMR)*

Nuclear magnetic resonance spectroscopy, as is implied in the name, involves the change of spin state of a nuclear magnetic moment when the nucleous absorbs electromagnetic radiation in a strong magnetic field. Two types of NMR spectroscopy in common use today are notably ^{1}H NMR and ^{13}C NMR.

The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometers operating at 400MHz at room temperature.The1HNMRspectra of the samples were recorded in $CDCl_3$, D_2O and Pyridine. The ^{1}H NMR spectra of the synthesized p-tert-butyl Calix[8]arene, of p-HCalix[8]arene of p-sulfonatocalix[8]arene, of [p-(N, N-Diallylaminomethyl)]calix[8]arene are shown in the figure 3.20, 3.24, 3.26, 3.28 respectively.

The ^{13}C NMR spectra of the samples were recorded in $CDCl_3$ as the standard. The ^{13}C NMR spectra of the synthesized p-tert-butylCalix[8]arene, of [p-(N, N-Diallylaminomethyl)]calix[8] arene in the figure 3.21, 3.29 respectively.

2.2.4 *Mass spectroscopy*

Mass spectroscopy is used to characterize organic molecule in two principle ways: (1) To measure exact molecular weights, and from this, exact molecular formula can be determined, (2) to indicate with in a molecule the points at which it prefers to fragment; from this, the presence of certain structural units in the organic compound can be recognized.

The LC-MS analysis was performed on a column Hypercil, C18, 5 μm 250x 4.6 nm at a flow rate of 1 ml/min (with Spliter). The LC-MS mass spectra of the parent p-tert-butyl calix[8]arene was shown in the figure 3.22.

2.2.5 *Fluorescence spectroscopy*

Fluorescence spectroscopy or fluorometry or spectrofluorimetry is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. It involves using a beam of light , usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light of a lower energy, typically, but not necessarily, visible light.

The fluorescence spectra were taken on a RF-5301PC spectrofluorometer at room temperature. A 450-W Xenon CW lamp was used as the excitation source. Slit width was set at 1.5 nm for excitation and emission respectively. The scan rate was $1nms^{-1}$. The fluorescence spectra of the water-soluble [60]fullerene –lysozyme adduct were shown in the figures 3.1 (a & b)

2.2.6 *Thermogravimetric Analysis*

It is a technique where by the weight of a substance, in an environment heated or cooled at a controlled rate, is recorded as a function of time or temperature. The TG curve obtained in each case will vary from instrument to instrument.

The Thermogram was taken on "SHIMADZU TGA50". The TGA of the of γ - cyclodextrin-[60]fullerene complex is shown in the Figure 3.42.