Cyclodextrin Covered Drug Nanomaterials - Evidence by Spectral, Electrochemical and Molecular Modeling Methods

Major Project Final Report submitted to

UNIVERSITY GRANT COMMISSION, NEW DELHI

F.No.41-351/2012 (SR) dated 13. 07. 2012

By

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UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI-110 002

Annual / Final Report of the work done on the Major Research Project
(Report to be submitted within 6 weeks after completion of each year)

1. Name and address of the Principal Investigator :

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2. Title of the project: Cyclodextrin Covered Drug Nanomaterials - Evidence by Spectral, Electrochemical and Molecular Modeling Methods

3. UGC approval no. and date : F.No. 41-351/2012(SR) dated: 13. 07. 2012

4. Date of implementation : 23. 07. 2012

5. Tenure of the project : 31. 12. 2015

6. Total grant allocated : **Rs. 11,84,800 /-**

7. i) Total grant received : **Rs. 7,55,800/-**

ii) Final expenditure : **Rs . 7,26,298/-**

iii) Balance amount : **Rs . 29,502/-**

8. Brief Objective of the Project:

Drug - cyclodextrin (α, and β) inclusion complex nanomaterials are fabricated and analysed by spectral, electrochemical and molecular modeling methods.

a) Nanomaterials in solution will be characterized by using UV- visible, fluorescence, time resolved fluorescence and cyclic voltammetry methods.

b) Nanomaterials in the solid state will be characterized by DSC, FT-IR, ^{1}H NMR and X-RD methods.

c) Structure of the nanomaterials (drug–cyclodextrin inclusion complex) will be theoretically calculated by molecular modeling methods (PM3) by using Gaussian software and Gauss view package.

d) Structure and morphology of nanomaterials will be characterized by Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM).

9) Methods and techniques to be used

A) Materials: It is proposed to make inclusion complex of many drugs in α-CD and β-CD and analysed. The drugs are purchased from different pharmaceutical industries or Sigma Aldrich chemical company**.**

B) Cyclodextrin study: All the inclusion complex nanomaterials are prepared by mixing different concentrations of cyclodextrin and the corresponding drug molecules, using appropriate solvents/ H_2O.

C) Techniques for characterization of inclusion complex nanomaterials

i) Characterization of nanomaterials in solution

UV- Visible Spectroscopy, Fluorescence Spectroscopy, Time Resolved Fluorescence Spectroscopy,

ii) Characterization of nanomaterials in the solid state

Differential Scanting Colorimeter, FT-IR Spectroscopy, NMR Spectroscopy, X-Ray Diffractiometry

iii) Characterization of Inclusion complex by molecular modeling methods

The structure of the drug–cyclodextrin inclusion complex thermodynamic parameters will be analysed by semiempirical quantum mechanical calculation - PM3 or DFT methods using Gaussian 09W software.

iv) Inclusion complex nanomaterials

The structure of the nanomaterials find out by Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) methods.

10. Summary of the findings (in 500 words)

The inclusion complexation between various drugs with α-CD and β-CD were carried out by UV–visible, steady state and time-resolved fluorescence, FT-IR, DSC, ^{1}H NMR, XRD, SEM, TEM and semi-empirical method (PM3). Some of the drug molecules are gave single emission maximum in water and CD and some of the drugs gave dual emission in the water and CDs. CDs study revealed that the drugs molecules were formed 1:1 or 1:2 inclusion complex. The hydrogen bonding and van der Waals interaction between the drugs and the CD plays an important role in the inclusion complexes.

The experimental results revealed that the inclusion process is a spontaneous process. Time-resolved fluorescence studies suggested that drugs exhibited biexponential decay in aqueous and triexponential decay in CD and significant enhancement of lifetime of decay components was observed in the CD solution. Morphologies of drug–CD complexes observed by SEM and TEM demonstrate that drugs are formed different nano-sized particles, or nanorods, or nanowire or nanomaterials with α-CD and β-CD.

The complexation energy, thermodynamic parameters and HOMO–LUMO, chemical potential (μ), stability (*S*), dipole moment, hardness (η), electrophilicity (ω) and Mulliken charge of the drugs, host (α-CD and β-CD) and the inclusion complexes were calculated using PM3 method. In general, drug:β-CD complex having high HOMO and LUMO energy gap indicate that this complex is more stable than the other complexes. The geometrical parameters like bond distance, bond angles and dihedral angles of the guest molecule changed before and after complexation suggesting that the guest molecule adopts a specific conformation leading to the formation of stable complexes.

11) If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the commission

1. *Fabrication of 2D nano sheet through self assembly behavior of sulfamethoxy pyridazine inclusion complex with β-cyclodextrin*

A 2D nano sheet was fabricated through the supramolecular self assembly of sulfamethoxypyridazine (SMP) and β-cyclodextrin (β-CD) inclusion complexes as building block units. The HRTEM image exhibited 2D nano sheet morphology with length of 1200 nm and the sheet thickness of 60 nm. From the HRTEM, it is noted that the nano sheet did not form a single layer aggregation but a bulk aggregation of SMP/β-CD inclusion complex. The formation of this multilayer 2D nano sheet based on the self assembly of SMP/β-CD inclusion complexes is proposed by the topological transformation as well as molecular modeling calculations. But, the SMP/α-CD inclusion complex self assembled to form nano rods, indicated that the nature of the CD determined the shape of the self assembled supramolecular architecture. The intermolecular hydrogen bonding and van der Waals forces are the main dominant driving forces for the construction of this type of supramolecular architecture. In order to confirm the interactions between SMP and CDs a detailed characterization was carried out by using FT-IR, P-XRD, DSC, ^{1}H-NMR, absorption spectroscopy, fluorescence emission spectroscopy and life time measurements.

2. *Preparation and Characterization of Sulfadimethoxine/Cyclodextrins Nanoparticles and Molecular Modeling Study*

Nano-encapsulation of sulfadimethoxine (SDMO) with α- and β-cyclodextrins (α-CD and β-CD) were carried out and the interactions investigated by UV-absorption, fluorimetry, ^{1}H NMR, FT-IR, DSC, powder PXRD, SEM, TEM and molecular modeling (PM3) methods. The SDMO molecule in the formation of encapsulated complexes with α-CD and β-CD to self-assemble into nano particles in water were observed by TEM. The absorption and fluorescence studies suggested that SDMO forms 1:1 encapsulated complexes with CDs. The spectral shifts reveals that the part of pyrimidine and aniline ring along with amide group is entrapped in the hydrophobic part of the CD nano-cavity and LW emission originates from the TICT state. PM3 calculations were performed for the possible

mode of encapsulation process of drug with CDs and are well consistent with the experimental results.

3. ***Nanostructures formed by Cyclodextrin covered aminobenzophenones through supramolecular self assembly***

Cyclodextrin based various type of nano structures produced from host:guest (2-aminobenzophenone, 3-aminobenzophenone) complexes through the supramolecular self assembly studied by SEM, TEM, FT-IR, DSC, PXRD, ^{1}HNMR, absorption and fluorescence and time resolved spectroscopy. The unequal layer by layer nanosheet and nano ribbon are formed through self assembly of numerous nanorods of 3ABP/CD complexes, the 2ABP with α-CD complex nanostructures show the self assembly hierarchical thread structure and β-CD complexes displays a nanobrick structure. The formation of nano structures are prearranged to OH---H, $NH_2\cdots O$ and $H_2N\cdots H$ intermolecular hydrogen bond between individual complex. Further the absorption and fluorescence spectroscopy changes explicit formation of 1:1 inclusion complexes and solvent study demonstrate the ESIPT and TICT in both molecules. The thermodynamic parameters (ΔH, ΔG and ΔS) of 2ABP and 3ABP molecule encapsulation process were determined from semiempirical PM3 calculations.

4. ***Nano chain and vesicles formed by inclusion complexation of 4, 4'-diaminobenzanilide with cyclodextrins***

The nano chain like agglomerates and spherical nano vesicles were formed from supramolecular self-assembly of 4,4'-diaminonenzanilide (DABA) with both α- and β-cyclodextrins. The inclusion complexes of DABA with α-CD and β-CD nanomaterials were prepared and characterized by TEM, SEM, FTIR, DSC, ^{1}H NMR and PXRD. ^{1}HNMR analysis indicated that the benzamido ring of DABA was encapsulated in to the CD cavity. Absorption and fluorescence spectral studies suggested DABA form different types of nanomaterials in α-CD and β-CD solutions. Theoretical studies of HOMO, LUMO, ΔE, ΔH, ΔG, ΔS and dipole moment values suggested that DABA encapsulate in the both CD cavities. In both CD, DABA form different type of 1:1 inclusion complex nanomaterials. Further, theoretical studies suggest that hydrophobic interaction plays an important role in determining the stability of the inclusion complexes.

The TEM exploration of the DABA/α-CD inclusion complex nanomaterials visualized nano chain like agglomerates with thickness of 25-100 nm and DABA/β-CD inclusion complex nanomaterials showed spherical nano vesicular structure with the diameter of 60-120 nm. The model proposed head-head and head-tail structure of DABA/α-CD and the secondary self assembly made the vesicular structure of DABA/β-CD. The results of DABA/α-CD and DABA/β-CD nanomaterials have different physicochemical characteristics from isolated DABA.

5. ***Nanorod formation of cyclodextrin-covered sudan dyes through supramolecular self-assembly***

Cyclodextrin-covered sudan III (SDIII) and sudan IV (SDIV) dyes produced various nanostructures such as pseudorotaxanes through supramolecular self-assembly, studied by absorption, fluorescence, time-resolved fluorescence, SEM, TEM, FT-IR, DSC, PXRD and 1H NMR. Solvent study shows that azo–hydrazo tautomer is present in sudan dyes. Absorption and fluorescence spectroscopy data gave evidence for the formation of 1:2 inclusion complexes. Big nanorod (36 nm) surrounded by small nanorod (3 nm) was identified in SDIV/a-CD complexes. This confirms that the rigid molecular nanorod aggregates of a-CD and b-CD complexes are formed through the initial formation of smaller nanorods. An unequal morphology noticed in SDIII/CD suggests that the 1:2 inclusion complexes were self-assembled into irregular arrangement. The thermodynamic parameters (ΔH, ΔG and ΔS) of inclusion processes were determined from semi-empirical PM3 calculations.

6. ***Inclusion complex of sulfadimethoxine with cyclodextrins:Preparation and characterization***

The inclusion complexation behavior, characterization and binding ability of sulfadimethoxine (SDMO) with cyclodextrin (α-CD and β-CD) have been investigated both in solution and solid state by means of absorption, fluorescence, time-resolved fluorescence,1H NMR, FT-IR, DSC, SEM, TEM,XRD and molecular modeling methods. The spectral shifts revealed that the part of pyrimidine and aniline rings of SDMO are entrapped in the CD cavity. The stoichiometric ratio and association constant were determined by Benesi–Hildebrand plots and spectroscopic studies respectively. FT-IR spectroscopy was used to compare inclusion systems with physical mixtures, and demonstrated the complex formation inthe solid state. The morphology and size of the nanoparticles of SDMO/CD complexes in aqueous solution were observed by TEM. The DSC analysis showed that the thermal stability of SDMO was enhanced in the presence of CD. Investigations of energetic and thermodynamic properties by PM3 method confirmed the stability of the inclusion complexes.

7. ***Host–guest inclusion complex of propafenone hydrochloride with a- and b-cyclodextrins: Spectral and molecular modeling studies***

Host–guest inclusion complexes of cyclodextrins (CDs) with a potential cardiovascular drug propafenone hydrochloride (PFO), were prepared and characterized using absorption, fluorescence, time-resolved fluorescence, SEM, FT-IR, DSC, 1H NMR, XRD and PM3 methods. The spectral studies suggested the phenyl ring along with carbonyl group is present inside of CD cavity. Solvent studies revealed that the normal Stokes shifted band originates from the locally excited state and the large Stokes shifted band occurs due to the emission from ICT. Nanosecond time-resolved studies indicated that PFO exhibits biexponential decay in water and triexponential decay in CD, indicating the formation of 1:1 inclusion complex. The results from solid state studies showed important modifications in the physicochemical properties of free PFO. The DH, DG and DS of the complexation process were determined and it was found that the complexation processes were

spontaneous. Investigations of thermodynamic and electronic properties confirmed the stability of the inclusion complex.

8. ***Inclusion complexation of sulfapyridine with a- and b-cyclodextrins: Spectral and molecular modeling study***

The inclusion complexes of sulfapyridine (SFP) with a-CD and b-CD were investigated by absorption, fluorescence, time-resolved fluorescence, FTIR, DSC, XRD, 1H NMR, SEM, TEM and molecular modeling methods. The normal fluorescence takes place from locally excited (LE) state while twisted intramolecular charge transfer (TICT) is responsible for highly Stokes shifted fluorescence. The enhancement of TICT emission in both CDs suggesting that the inclusion process plays the major role in this emission. The spectral shifts revealed that part of pyridine ring of SFP is entrapped in the CDs cavities. TEM images confirmed round shaped nanoparticles with the average size about 20–50 nm were observed in SFP with a- CD and b-CD inclusion complexes. PM3 calculations have suggested that the large stabilization of excited singlet state of SFP with twisted conformation occurring at the amide SAN bond between the electron donor group (aniline ring) and the electron acceptor group (pyridine ring).

9. ***Inclusion complexation of isoprenaline and methyl dopa with a- and β-cyclodextrin nanocavities: Spectral and theoretical study***

Inclusion complex formation of isoprenaline (ISOP) and methyldopa (MDOP) with a-CD and b-CD were investigated. Solid inclusion complex nanomaterials were characterized by SEM, TEM, FTIR, DSC, 1H NMR and XRD methods. Spectral results showed that single emission (monomer) noticed in aqueous solution where as dual emission (excimer) in CD. Both drugs formed 1:2 (CD–drug2) inclusion complexes with CDs. Time-resolved fluorescence studies show that single exponential decay observed in water whereas biexponential decay observed in CD. Nano-sized particles were found in ISOP/CD while vesicles were obtained in MDOP/CD complexes. DSC results revealed that the thermal stability of drugs was improved when it was included in the CD nanocavity. Based on PM3 calculations, the inclusion structure of ISOP/CD and MDOP/CD complexes were proposed. Thermodynamic parameters and binding affinity of complexation of CD were determined by PM3 method.

10. ***A spectroscopic and molecular modeling studies of the inclusion complexes of orciprenaline and terbutaline drugs with native and modified cyclodextrins***

The inclusion complexation behavior of orciprenaline (ORC) and terbutaline (TER) with a-CD, b-CD, HP-a-CD and HP-b-CD are examined by absorption, fluorescence, life time and molecular modeling methods. ORC and TER forms 1:1 (CD/drug) inclusion complexes in lower CD concentrations and 1:2 (CD/drug) inclusion complexes with higher CD concentrations. The inclusion of both drugs with HP-CDs was stronger than that of native CDs. Both drugs exhibit dual emission (excimer) in the CD solution, whereas in water single emission is seen. The hydrogen bonding and

van der Waals interaction between the drugs and the CD plays an important role in the inclusion complexes. Computational results show the side chain of the drugs encapsulated in the CD cavity. The molecular modeling results by PM3 were in good agreement with the experimental results.

12) Any other information which would help in evaluation of this work done of the project. At the completion of the project, the final report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

A) I wish to inform you Sir that the UGC has sanctioned the above titled project for my research. UGC have been conducted the Project Midterm review meeting from 01.02.2014 to 08.02.2014 and released the Expert Committee's Recommendation about the progress of the project in the website (UGC web site, Science Midterm review report): (**a)** From our University, ***56 staff members*** were attended the review meeting. The UGC has given excellent grade only for my project work. (**b)** In the Chemistry subject, ***169 staff members*** were attended the review meeting from various Universities and Colleges (all over India). Out of which, three project works only recommended as excellent including mine. (**c)** Further, in all the subjects (Social Sciences -***716***, Sciences, Agriculture, Engineering, and Medicines -***1296***) total- ***2012 staff members*** were attended the review meeting from various Universities and Colleges (all over India). ***Out of which, seven project works only recommended as excellent including mine.***

B) No. of publications out of the project

Articles published -45 **Conference – 23**

N. Rajendiran

Principal Investigator

Registrar

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