Summary of Thesis

# Development of Control Drug Delivery Systems Based on Mesoporous Silica


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*By*

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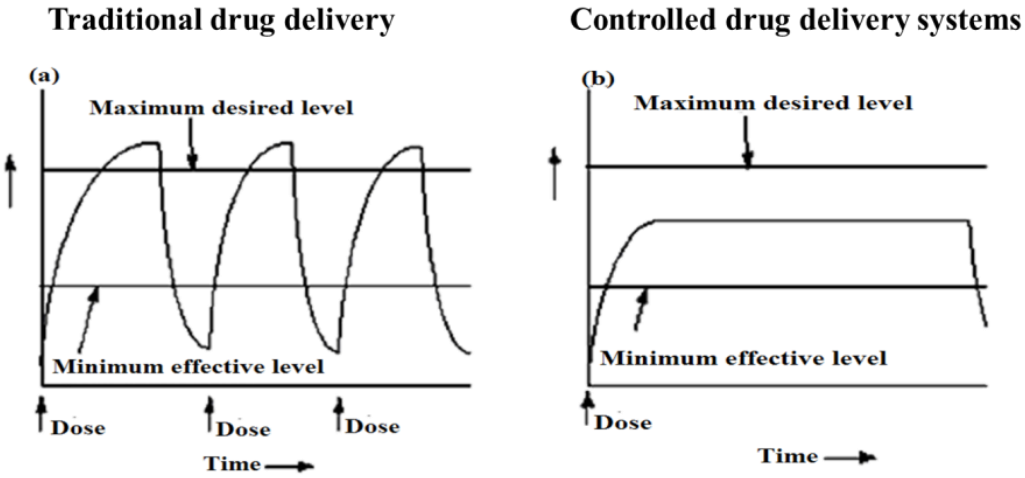
## Introduction

Control drug delivery system can be described as formulation that controls the rate and period of drug delivery that is time release doses. Control drug delivery system can deliver precise quantities of therapeutic drugs in tailored release manner to enhance drug efficiency and reduce toxicity. The conventional dosage forms provide drug release immediately and it causes fluctuation of drug level in blood depending upon dosage form. The drug delivery system should efficiently encapsulate drugs at high concentration and release it in controlled manner for a prescribed period of time.




Scheme 1. Release profile of drug in traditional and controlled drug delivery systems

Hydrogels, Liposomes, polymeric micelles and vesicles are some of the example of drug delivery systems which have been earlier used. However, they have some limitations like Poor chemical stability, rapid elimination by body functioning processes, lack of homogeneous distribution of drug which affects the release rate. Further, specifically designed macroscopic and microscopic structural and chemical features are lacking in polymers.

The ordered mesoporous silica (MCM-41 and MCM-48) is consider as very good drug delivery system because of their typical characteristics, such as highly regular pore structure, uniform pore size and high surface area. The pore size and pore volume can be tailored selectively to bind molecules of various size. There are abundant Si-OH groups on interior and outer surface of materials which can be functionalized for better control over the drug diffusion kinetics [1]. Numbers of reports are available on mesoporous silica functionalized by organic moiety such as Long alkyl chain containing amino group, carboxylic group and thiol group, etc.

So it was a thought of interest to use polyoxometalates, mainly 12-tungstophosphoric acid for functionalisation instead of traditional organic moiety. Polyoxometalates are early transition metal oxygen anion clusters with metal in their higher oxidation state like W (VI), Mo (VI), V(V). They show the property of multifunctionality, structural mobility and easy alteration of chemical composition. Cysteine, L-arginine, Aspirin, Captopril and Camptothecin are selected for the study, because of the following reasons.

Cysteine is a sulfur-based amino acid, cysteine itself can act as an antioxidant in the body, its pro-drugs are used to treat Schizophrenia and reduce drug cravings [2]. It is a limiting substrate in the production of glutathione in the body. Current cysteine therapies are administration of different cysteine derivatives such as N-acetylcysteine. One of the major drawbacks of these therapies is high dosages that can provoke persistent damage and strong allergic reactions [3-6].

L-arginine intravenously administered to the patients with coronary artery disease to increase vascular nitric oxide (NO) bioavailability which show the vasodilatory effect. But the oral administration of arginine does not show this effect. So oral administration of L-arginine via drug delivery system using MCM-41 can overcome this problem and provide L-arginine for NO production [7,8].

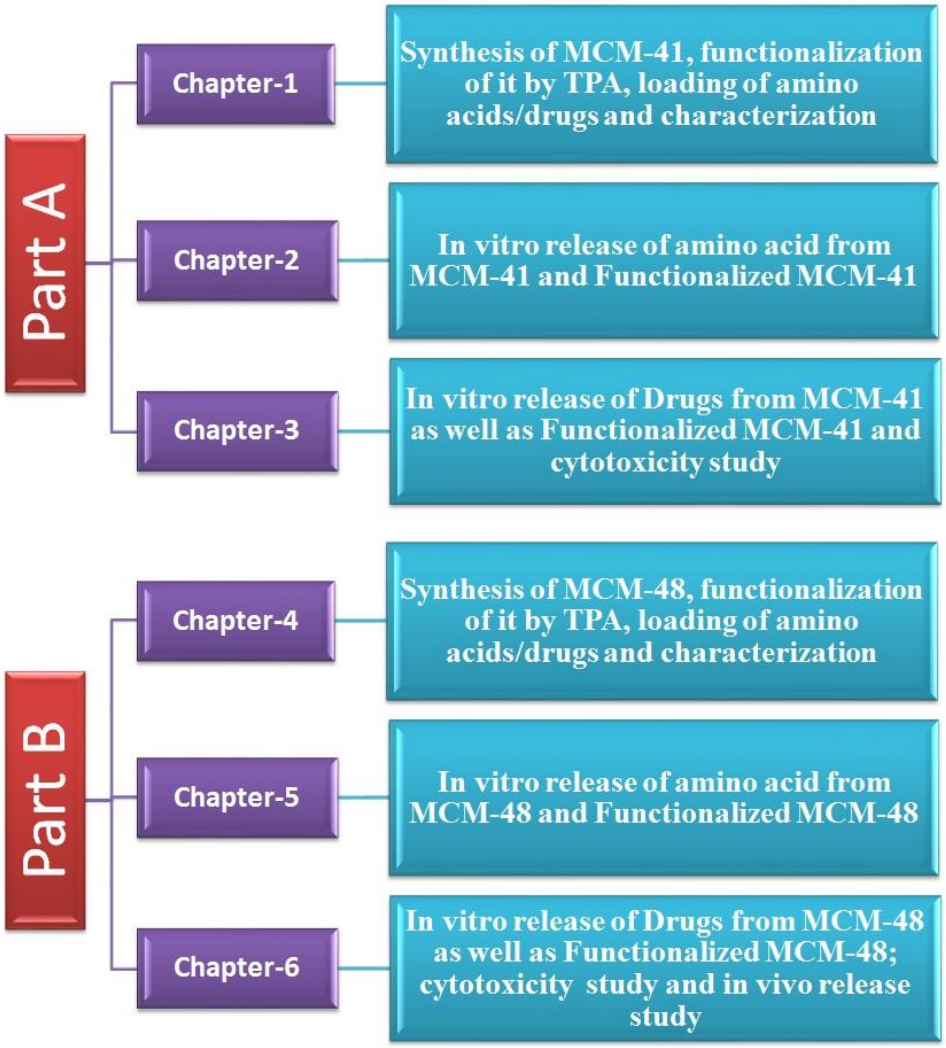
A poorly-water soluble drug, aspirin is primarily used for the treatment of cardiovascular diseases1 and as a non-steroidal anti-inflammatory drug which acts by inhibiting the enzyme cyclooxygenase. Captopril is water soluble drug and used in the treatment of controlling blood pressure, heart failure, preventing cardiac remodeling and left ventricular dysfunction after myocardial infection, and preventing kidney damage in human with hypertension and diabetes [9,10]. The elimination half -life of all this drugs is very short which can be overcome using drug delivery system. Camptothecin (CPT) is a naturally occurring quinolone alkaloid which shows significant anticancer activity with a broad spectrum of human malignancies and CPT is an inhibitor of the DNA-replicating enzyme topoisomerase-I [11]. Unfortunately, the clinical application of CPT is hindered by its poor pharmaceutical profile, with extreme aqueous insolubility, low stability of the lactone form at physiological pH, and severe systemic toxicities which included myelosuppression, vomiting, diarrhoea, and hemorrhagic cystitis [12-16]. Therefore, the

development of controlled delivery strategies could lead to significant advantages in the clinical use of these drugs.

The objectives have been planned as follows.

- To develop controlled drug delivery system based on mesoporous silica (MCM-41 and MCM-48) and polyoxometalates, 12-tungstophophoricacid.
- To synthesize MCM-41 and MCM-48, to characterize them using various techniques such as XRD, SEM, TEM, FTIR, NMR, TG-DTA, BET analysis as well as to load L-Arginine, Cysteine, Captopril, Aspirin and Camptothecin and to characterize them using same techniques.
- To study the in vitro amino acids/drugs release in simulated body fluid (similar to blood plasma pH 7.4) and gastric fluid (pH 1.2). Amino acids/drugs release will be monitored using UV-Visible spectrometry, under stirring and static condition.
- To functionalize MCM-41 and MCM-48 using 12-tungstophosphoricacid (TPA), loading with the mentioned amino acids/drugs and their characterization.
- To see the effect of functionalization on release profile of drug molecules.
- To study the kinetics and release mechanism of drugs using different models such as $1^{st}$ order release kinetic model, Higuchi model, Korsmeyer-Peppas model (KPM) and Extended kinetic model (EKM).
- MTT study for Camptothecin loaded systems.
- To study the in vivo Camptothecin release from the selected best systems.
- To design MCM-41 type mesoporous silica nanoparticle and study is application as carrier.

The thesis is comprised of general introduction, six chapters and annexure section. Part A consists three chapters and Part B consists three chapters.




**Part A** describes synthesis of MCM-41, its functionalization by TPA, loading of Amino acids/drugs into the same and in vitro release.

**Chapter 1A** describes Synthesis of MCM-41 and loading of Amino acids/drugs and characterizations. Loading of L-arginine was carried out by wet impregnation and Cysteine by wet impregnation as well as by soaking method. Loading of all drugs were carried out by soaking method. The prepared materials were characterized by various physicochemical techniques such as Thermo Gravimetric Analysis (TGA), Fourier Transform Infrared Spectroscopy (FTIR), Surface Area Measurement (BET method), Pore Size, Pore Volume X-ray Diffraction (XRD), and $^{29}Si$ MAS-NMR. Further, the surface morphology was studied by Transmission Electron Microscopy (TEM).

**Chapter 1B** describes functionalization of MCM-41 by wet impregnation method using 12-tungstophopsphoric acid (TPA-MCM-41) as well as loading of Amino acids/drugs and their characterizations using same techniques as stated above.

**Chapter 2** describe the in vitro release study as well as kinetics and mechanism of L-Arginine and Cysteine from MCM-41 and TPA-MCM-41.

Release study of L-Arginine was carried out under different conditions such as static and stirring as well as at different pH. The obtained results suggest that under static condition slow release was observed for L-Arginine which may be due to the slower diffusion molecules from the carrier (Table 1). Further, study shows that pH has great influence on release rate. In acidic pH slow release was observed for L-Arginine, as it acquires two positive charges and remain as $Arg^{2+}$. These may have strong interaction with terminal oxygen of TPA moiety which will slow down the diffusion of L-Arginine molecules.

To see the effect of TPA on release rate of L-arginine, release profile obtained from MCM-41 and TPA-MCM-41 has been compared (Table 1). The results shows that L-Arginine loaded into TPA-MCM-41 shows more controlled and delayed release as compared to that of pure MCM-41. As TPA has terminal free oxygen through which it can bind with amino acids and hold it for longer period of time.

**Table 1.** In vitro release profile of L-arg/MCM-41 and L-arg/TPA-MCM-41

| Materials | Conditions | % Release | | |
|---|---|---|---|---|
| | | Initial | After 10h | 30 h |
| **L-arg/MCM-41** | stirring | 35 | 67 | 91 |
| | Static | 30 | 62 | 86 |
| **L-arg/MCM-41** | SBF (pH 7.4) | 35 | 67 | 91 |
| | SGF (pH 1.2) | 28 | 48 | 65 |
| **L-arg/TPA-MCM-41** | stirring | 25 | 56 | 80 |

To investigate the release Kinetic and mechanism First order release kinetic model and Higuchi Model has been applied (Table 2, Figure 1 and Figure 2). All the release data were fitted to the model and results shows that L-arg/TPA-MCM-41 was best fitted to these model and follows first order release kinetic model and Higuchi Model

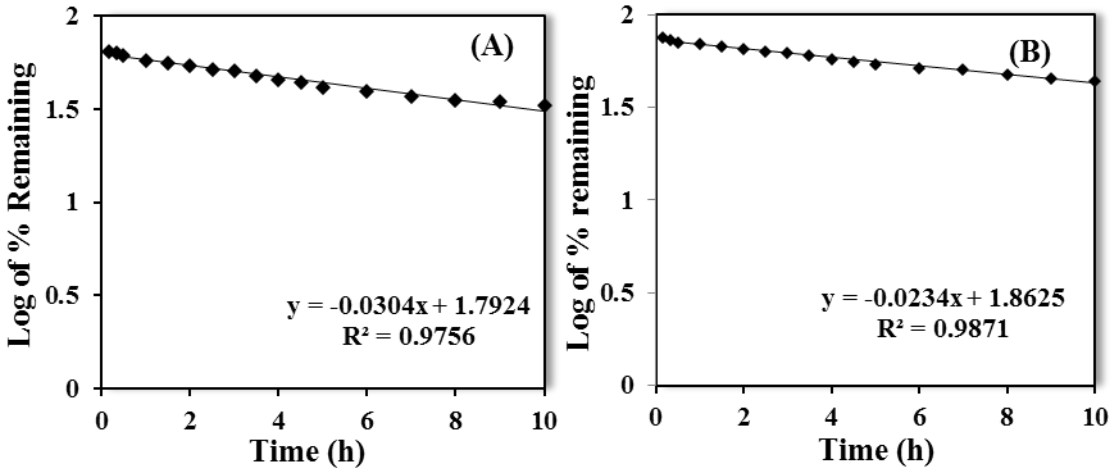



**Figure 6.** First order release kinetic model for (A) L-arg/MCM-41 and (B) L-arg/TPA-MCM-41

**Table 2.** Kinetic parameters obtained from First order release kinetic model and Higuchi model

| Model | L-arg/MCM-41 | L-arg/TPA-MCM-41 |
|---|---|---|
| **First order release kinetic model** | $K_1$ = 0.030<br>$R_1$ = 0.975 | $K_1$ = 0.0234<br>$R_1$ = 0.987 |
| **Higuchi model** | $K_2$ = 11.95<br>$R_2$ = 0.990 | $K_2$ = 11.43<br>$R_2$ = 0.991 |

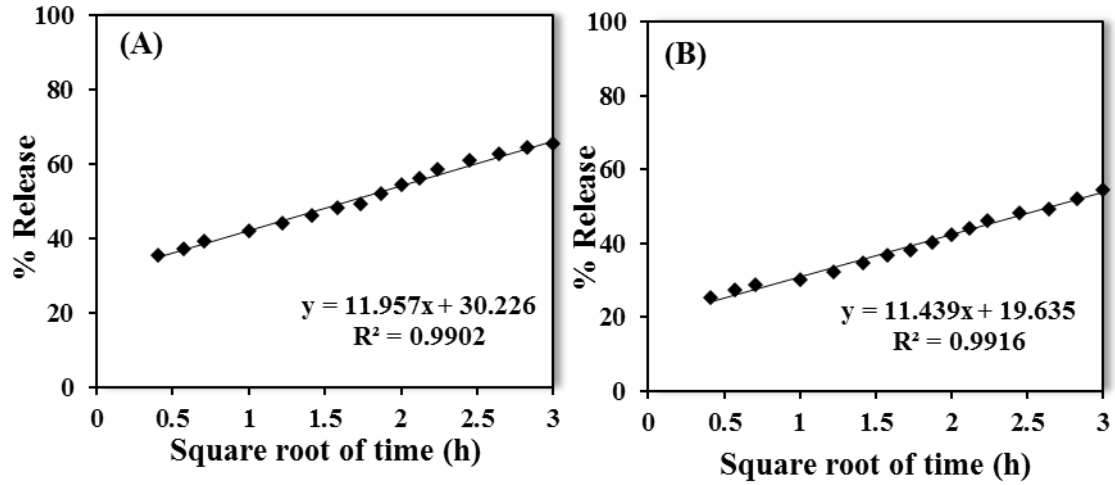



**Figure 2.** Higuchi Model for (A) L-arg/MCM-41 and (B) L-arg/TPA-MCM-41

Cysteine loaded into MCM-41 by wet impregnation method (Cys/MCM-41(I)) and soaking method (Cys/MCM-41). Comparison of release profile of Cys-MCM-41(I) and Cys-MCM-41 shows that more controlled release was observed for later case (Table 3). The reason behind is that most of the Cysteine molecules are adsorbed on the outer surface

of MCM-41 with relatively low concentration of Cysteine inside the channels of MCM-41. As a consequence, burst and fast release of loosely held Cysteine on the outer surface of MCM-41 is observed for Cys-MCM-41(I) (Table 3).

To see the effect of string release study was carried out under string and static condition and results displays that lower release under static condition (Table 3). Further, to see the effect of TPA on release rate, release profile of Cys-MCM-41 and Cys-TPA-MCM-41 has been compared and result shows that more ordered and controlled release was observed for Cys-TPA-MCM-41 system.

**Table 3.** In vitro release profile of Cys-MCM-41(I), Cys-MCM-41 and Cys-TPA-MCM-41

| Materials | Conditions | % Release | | |
|---|---|---|---|---|
| | | Initial | After 10h | 30 h |
| **Cys-MCM-41(I)** | Stirring | 60 | 82 | 98 |
| **Cys-MCM-41** | Stirring | 38 | 72 | 91 |
| | Static | 30 | 51 | 80 |
| **Cys-TPA-MCM-41** | Stirring | 30 | 61 | 84 |

To understand the Cysteine release kinetic and mechanism First order release kinetic model and Higuchi Model were applied (Table 4) and result shows that Cys-TPA-MCM-41 system was best fitted to both model as well as it follows the First order release kinetic model and diffusion mechanism.

**Table 4.** Kinetic parameters obtained from First order release kinetic model and Higuchi model

| Model | Cys-MCM-41(I) | Cys-MCM-41 | Cys-TPA-MCM-41 |
|---|---|---|---|
| **First Order Release Kinetic model** | $K_1 = 0.0363$<br>$R_1 = 0.936$ | $K_1 = 0.0328$<br>$R_1 = 0.986$ | $K_1 = 0.0232$<br>$R_1 = 0.988$ |
| **Higuchi model** | $K_2 = 7.83$<br>$R_2 = 0.963$ | $K_2 = 11.67$<br>$R_2 = 0.9955$ | $K_2 = 11.353$<br>$R_2 = 0.9959$ |

**Chapter 3** describe the in vitro release study as well as kinetics and mechanism of Captopril, Aspirin and Camptothecin from MCM-41 and TPA-MCM-41. Release study of all the drugs were carried out under string and static condition as well as at different pH (Table 5). The obtained result shows that under static condition slower release was observed for all the drugs. Further, it shows that in acidic pH (1.2) release rate became fast as compared to that obtained in pH 7.4 (Table 5).

**Table 5.** In vitro release profile of all the drugs from MCM-41 and TPA-MCM-41

| Materials | Conditions | %Release | | |
|---|---|---|---|---|
| | | Initial | After 10h | 30 h |
| **Cap/MCM-41** | stirring | 44 | 83 | 96 up to 27h |
| | Static | 38 | 56 | 66 |
| **Cap/TPA-MCM-41** | stirring | 34 | 67 | 89 |
| | Static | 18 | 32 | 37 |
| **Cap/MCM-41** | SBF (pH 7.4) | 44 | 83 | 96 |
| | SGF (pH 1.2) | 53 | 93 | 96 up to 16 h |
| **Cap/TPA-MCM-41** | SBF (pH 7.4) | 34 | 67 | 89 |
| | SGF (pH 1.2) | 38 | 73 | 90 up to 20 h |
| **Asp/MCM-41** | stirring | 41 | 61 | 96 up to 28 h |
| | Static | 12 | 15 | 24 |
| **Asp/TPA-MCM-41** | stirring | 25 | 56 | 89 |
| | Static | 10 | 14 | 24 |
| **Asp/MCM-41** | SBF (pH 7.4) | 41 | 61 | 96 up to 28 h |
| | SGF (pH 1.2) | 47 | 88 | 97 up to 18 h |
| **Asp/TPA-MCM-41** | SBF (pH 7.4) | 25 | 56 | 89 |
| | SGF (pH 1.2) | 30 | 80 | 97 up to 25 h |
| **CPT/MCM-41** | stirring | 28 | 72 | 98 up to 28 h |
| | Static | 18 | 33 | 58 |
| **CPT/TPA-MCM-41** | stirring | 21 | 62 | 90 |
| | Static | 10 | 27 | 52 |

To see the effect of TPA on release rate of drugs, release profiles obtained from MCM-41 as well as TPA-MCM-41 has been compared and results shows that in later case more ordered and delayed release was observed (Table 5).

To investigate the drug release kinetic and mechanism release data were fitted to First ordered release kinetic model, Higuchi Model (Figure 4, 5) , Korsmeyer-Peppas model (KPM) and extended kinetic model (EKM). The obtained results shows that release of all the drugs follows first order release kinetic model and Fickian diffusion mechanism which was supported by KPM and EKM (Table 6).

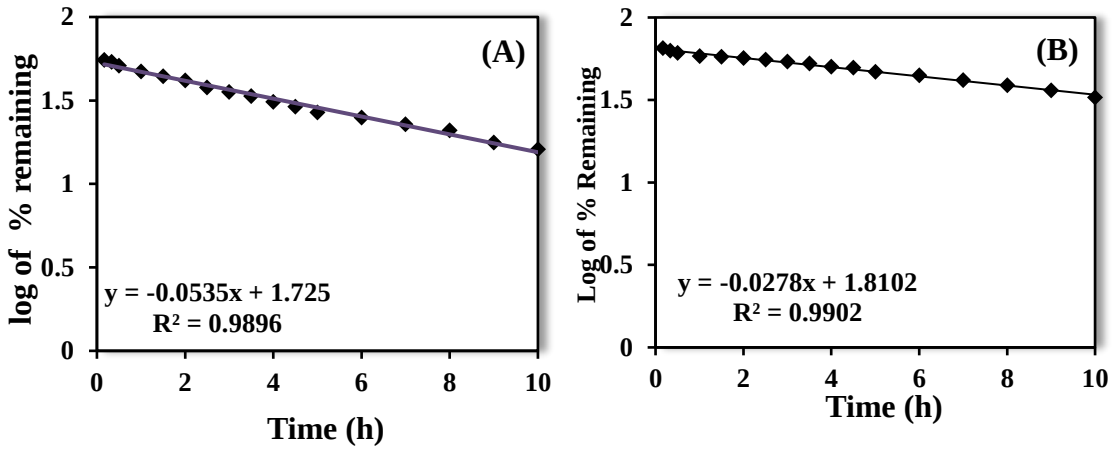



**Figure 7.** First order release kinetic model for (A) Cap/MCM-41 and (B) Cap/TPA-MCM-41

**Table 6.** Estimated parameters of first order release kinetic model, Higuchi model, KPM and EKM model for all drugs release from MCM-41 and TPA-MCM-41. Units: $k_1$, $k_2$ ($h^{-1}$ mL $mg^{-1}$); $K = k_2/k_1$

| Materials | Model | | | |
|---|---|---|---|---|
| | First order release kinetic model | Higuchi model | KPM | EKM |
| **Cap/MCM-41** | $K_1$ = 0.0535<br>$R_1$ = 0.986 | $K_1$ = 0.0278<br>$R_1$ = 0.990 | - | - |
| **Cap/TPA-MCM-41** | $K_2$ = 14.93<br>$R_2$ = 0.991 | $K_2$ = 10.91<br>$R_2$ = 0.993 | - | - |
| **Asp/MCM-41** | $K_1$ = 0.0174<br>$R_1$ = 0.954 | $K_1$ = 14.88<br>$R_1$ =0.979 | $k_1$= 0.0166<br>K= 5.60 | n = 0.47 |
| **Asp/TPA-MCM-41** | $K_2$ = 0.0211<br>$R_2$ = 0.966 | $K_2$ = 10.46<br>$R_2$ = 0.989 | $k_1$= 0.0103<br>K= 4.59 | n = 0.49 |
| **CPT/MCM-41** | $K_1$= 0.0383<br>$R_1$=0.9694 | $K_2$= 14.397<br>$R_2$= 0.9522 | $k_1$= 0.0061<br>K= 6.19 | n = 0.48 |
| **CPT/TPA-MCM-41** | $K_1$= 0.0302<br>$R_1$= 0.9775 | $K_2$= 14.793<br>$R_2$= 0.997 | $k_1$= 0.0105<br>K= 4.76 | n = 0.5 |

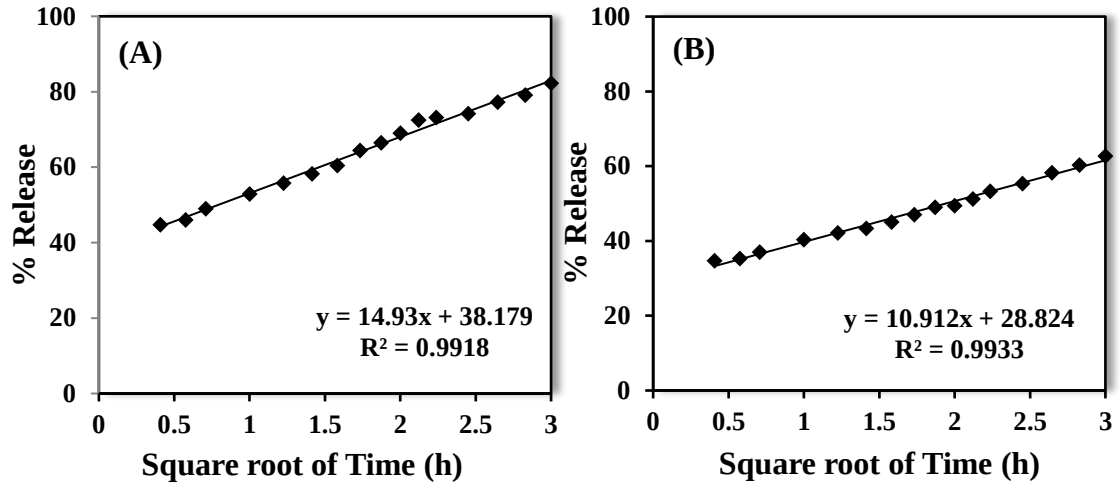



**Figure 4.** Higuchi Model for (A) Cap/MCM-41 and (B) Cap/TPA-MCM-41

To check whether the TPA induce toxicity into MCM-41 or not, cystotoxicty study was carried out and results shows that MCM-41 showed ≤ 10% cytotoxicity in the said doses (0.1, 0.3, 0.5 mg/ml, Figure 5). The functionalized material, TPA-MCM-41 recorded < 30% cytotoxicity whereas functionalized carrier loaded with drug CPT (CPT/TPA-MCM-41) showed > 40% at 0.5 mg/ml dose. Overall, the results indicate that the TPA-MCM-41 carrier is non-toxic to the cells but the drug loaded carrier accounts for highest percentage of cytotoxicity amongst all groups. These results imply towards CPT/TPA-MCM-41 mediated improved delivery of CPT that can be of significance in cancer therapy.

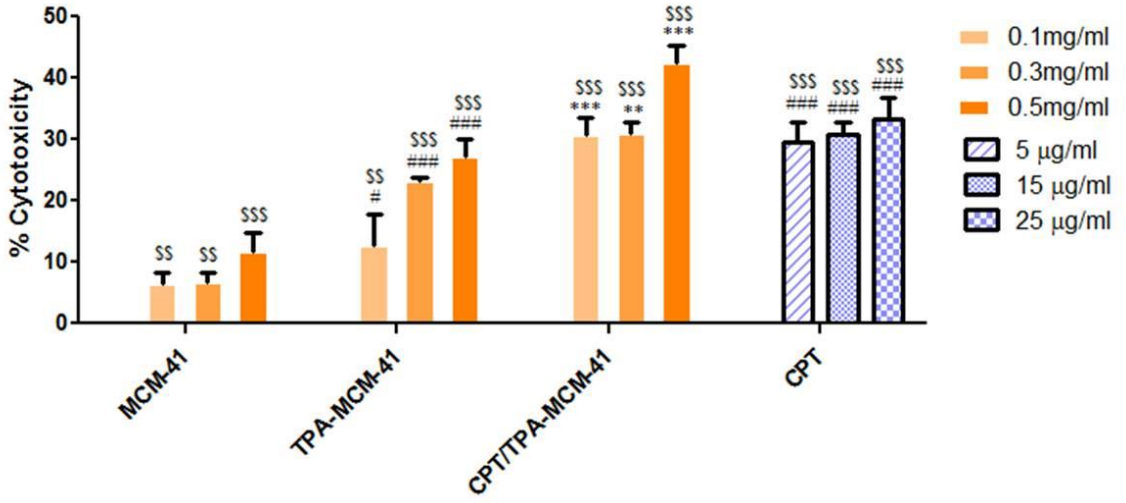



**Figure 5.** Effect of materials on the cytotoxicity of HepG2 cells. Control cells did not show cytotoxicity. Results are expressed as mean ± SD for n=3. $$P < 0.01 and $$$P < 0.001 as compared to control of respective groups and #P < 0.05, ###P < 0.001 as

compared to respective concentrations of MCM-41. **P < 0.01 and ***P < 0.001 as compared to respective concentrations of TPA-MCM-41.

**Part B** describes synthesis of MCM-48, its functionalization by TPA, loading of Amino acids/drugs into the same and in vitro release.

**Chapter 4A** describes Synthesis of MCM-48 and loading of Amino acids/drugs and characterizations. Loading of L-arginine was carried out by wet impregnation and Cysteine by wet impregnation as well as by soaking method. Loading of all drugs were carried out by soaking method. The prepared materials were characterized by various physicochemical techniques as mentioned in chapter 1A.

**Chapter 4B** describes functionalization of MCM-48 by wet impregnation method using 12-tungstophopsphoric acid (TPA-MCM-48) as well as loading of Amino acids/drugs and their characterizations using same techniques as stated in chapter 1A.

**Chapter 5** describe the in vitro release study as well as kinetics and mechanism of L-Arginine and Cysteine from MCM-48 and TPA-MCM-48.

Release study of L-Arginine was carried out under different conditions such as static and stirring as well as at different pH. The obtained results suggest that under static condition slow release was observed for L-Arginine which may be due to the slower diffusion molecules from the carrier (Table 7). Further, study shows that pH has great influence on release rate. In acidic pH slow release was observed for L-Arginine, as it acquires two positive charges and remain as $Arg^{2+}$. These may have strong interaction with terminal oxygen of TPA moiety which will slow down the diffusion of L-Arginine molecules.

**Table 7.** In vitro release profile of L-arg/MCM-48 and L-arg/TPA-MCM-48

| Materials | Conditions | % Release | | |
|---|---|---|---|---|
| | | Initial | After 10h | 30 h |
| **L-arg/MCM-48** | stirring | 52 | 69 | 87 |
| | Static | 24 | 58 | 79 |
| **L-arg/MCM-48** | SBF (pH 7.4) | 52 | 69 | 87 |
| | SGF (pH 1.2) | 34 | 51 | 71 |
| **L-arg/TPA-MCM-48** | stirring | 28 | 51 | 86 |
| | Static | 28 | 38 | 46 |
| **L-arg/TPA-MCM-48** | SBF (pH 7.4) | 28 | 51 | 86 |
| | SGF (pH 1.2) | 28 | 43 | 70 |

To see the effect of TPA on release rate of L-arginine, release profile obtained from MCM-41 and TPA-MCM-48 has been compared (Table 7). The results shows that L-Arginine loaded into TPA-MCM-48 shows more controlled and delayed release as compared to that of pure MCM-48. As TPA has terminal free oxygen through which it can bind with amino acids and hold it for longer period of time.

To investigate the release Kinetic and mechanism First order release kinetic model and Higuchi Model has been applied (Table 8). All the release data were fitted to the model and results shows that L-arg/TPA-MCM-48 was best fitted to these model and follows first order release kinetic model and Higuchi Model.

**Table 8.** Kinetic parameters obtained from First order release kinetic model and Higuchi model

| Model | L-arg/MCM-48 | L-arg/TPA-MCM-48 |
|---|---|---|
| **First order release kinetic model** | $K_1 = 0.021$<br>$R_1 = 0.982$ | $K_1 = 0.014$<br>$R_1 = 0.990$ |
| **Higuchi model** | $K_2 = 6.49$<br>$R_2 = 0.956$ | $K_2 = 7.70$<br>$R_2 = 0.986$ |

Cysteine loaded into MCM-48 by wet impregnation method (Cys/MCM-48(I)) and soaking method (Cys/MCM-48). Comparison of release profile of Cys-MCM-48(I) and Cys-MCM-48 shows that more controlled release was observed for later case (Table 9). The reason behind is that most of the Cysteine molecules are adsorbed on the outer surface of MCM-48 with relatively low concentration of Cysteine inside the channels of MCM-48. As a consequence, burst and fast release of loosely held Cysteine on the outer surface of MCM-48 is observed for Cys-MCM-48(I) (Table 3).

**Table 9.** In vitro release profile of Cys-MCM-41(I), Cys-MCM-41 and Cys-TPA-MCM-41

| Materials | Conditions | % Release | | |
|---|---|---|---|---|
| | | Initial | After 10h | 30 h |
| **Cys-MCM-48(I)** | Stirring | 51 | 82 | 97 |
| **Cys-MCM-48** | Stirring | 34 | 76 | 91 up to 26 h |
| | Static | 28 | 49 | 70 |
| **Cys-TPA-MCM-48** | Stirring | 28 | 59 | 91 up to 26 h |
| | static | 25 | 53 | 75 |

To see the effect of string release study was carried out under string and static condition and results displays that lower release under static condition (Table 3). Further,

to see the effect of TPA on release rate, release profile of Cys-MCM-48 and Cys-TPA-MCM-48 has been compared and result shows that more ordered and controlled release was observed for Cys-TPA-MCM-48 system.

To understand the Cysteine release kinetic and mechanism First order release kinetic model and Higuchi Model were applied (Table 10) and result shows that Cys-TPA-MCM-48 system was best fitted to both model as well as it follows the First order release kinetic model and diffusion mechanism.

**Table 10.** Kinetic parameters obtained from First order release kinetic model and Higuchi model

| Model | Cys/MCM-48 | Cys/MCM-48(I) | Cys/TPA-MCM-48 |
|---|---|---|---|
| **First order release kinetic model** | $K_1 = 0.045$<br>$R_1 = 0.994$ | $K_1 = 0.050$<br>$R_1 = 0.956$ | $K_1 = 0.021$<br>$R_1 = 0.996$ |
| **Higuchi model** | $K_2 = 15.60$<br>$R_2 = 0.991$ | $K_2 = 12.20$<br>$R_2 = 0.977$ | $K_2 = 10.79$<br>$R_2 = 0.996$ |

**Chapter 6** describe the in vitro release study as well as kinetics and mechanism of Captopril, Aspirin and Camptothecin from MCM-48 and TPA-MCM-48. Release study of all the drugs were carried out under string and static condition as well as at different pH (Table 11). The obtained result shows that under static condition slower release was observed for all the drugs. Further, it shows that in acidic pH (1.2) release rate became fast as compared to that obtained in pH 7.4 (Table 11).

To see the effect of TPA on release rate of drugs, release profiles obtained from MCM-41 as well as TPA-MCM-48 has been compared and results shows that in later case more ordered and delayed release was observed (Table 11).

**Table 11.** In vitro release profile of all the drugs from MCM-48 and TPA-MCM-48

| Materials | Conditions | %Release | | |
|---|---|---|---|---|
| | | Initial | After 10h | 30 h |
| **Cap/MCM-48** | stirring | 46 | 94 | 98 up to 25 h |
| | Static | 25 | 44 | 51 up to 25 h |
| **Cap/TPA-MCM-48** | stirring | 33 | 65 | 93 |
| | Static | 25 | 42 | 60 |
| **Cap/MCM-48** | SBF (pH 7.4) | 46 | 94 | 98 up to 25 h |
| | SGF (pH 1.2) | 50 | 98 up to 7 h | |
| **Cap/TPA-MCM-48** | SBF (pH 7.4) | 33 | 65 | 93 |
| | SGF (pH 1.2) | 45 | 87 | 98 up to 18 h |
| **Asp/MCM-48** | stirring | 43 | 80 | 97 |
| | Static | 12 | 19 | 24 |
| **Asp/TPA-MCM-48** | stirring | 38 | 71 | 81 |
| | Static | 10 | 19 | 24 |
| **Asp/MCM-48** | SBF (pH 7.4) | 43 | 80 | 97 |
| | SGF (pH 1.2) | 49 | 99 | - |
| **Asp/TPA-MCM-48** | SBF (pH 7.4) | 38 | 71 | 81 |
| | SGF (pH 1.2) | 44 | 95 | 98 up to 12 h |
| **CPT/MCM-48** | stirring | 36 | 74 | 98 up to 26 h |
| | Static | 27 | 42 | 55 |
| **CPT/TPA-MCM-48** | stirring | 30 | 68 | 97 |
| | Static | 18 | 38 | 54 |

To investigate the drug release kinetic and mechanism release data were fitted to First ordered release kinetic model, Higuchi Model, Korsmeyer-Peppas model (KPM) and extended kinetic model (EKM). The obtained results shows that release of all the drugs follows first order release kinetic model and Fickian diffusion mechanism which was supported by KPM and EKM (Table 12).

**Table 12.** Estimated parameters of first order release kinetic model, Higuchi model, KPM and EKM model for all drugs release from MCM-48 and TPA-MCM-48. Units: $k_1$, $k_2$ ($h^{-1}$ mL $mg^{-1}$); $K = k_2/k_1$

| Materials | Model | | | |
|---|---|---|---|---|
| | **First order release kinetic model** | **Higuchi model** | **KPM** | **EKM** |
| **Cap/MCM-48** | $K_1 = 0.092$<br>$R_1 = 0.973$ | $K_1 = 15.77$<br>$R_1 = 0.943$ | - | - |
| **Cap/TPA-MCM-48** | $K_2 = 0.030$<br>$R_2 = 0.974$ | $K_2 = 12.00$<br>$R_2 = 0.981$ | - | - |
| **Asp/MCM-48** | $K_1 = 0.054$<br>$R_1 = 0.9614$ | $K_1 = 14.88$<br>$R_1 = 0.979$ | $k_1 = 0.0044$<br>$K = 9.0 * 10^{-2}$ | n = 0.48 |
| **Asp/TPA-MCM-48** | $K_2 = 0.029$<br>$R_2 = 0.9707$ | $K_2 = 10.40$<br>$R_2 = 0.983$ | $k_1 = 0.084$<br>$K = 6.3 * 10^{-2}$ | n = 0.5 |
| **CPT/MCM-48** | $K_1 = 0.038$<br>$R_1 = 0.969$ | $K_2 = 13.84$<br>$R_2 = 0.9905$ | $k_1 = 0.0059$<br>$K = 5.75$ | n = 0.49 |
| **CPT/TPA-MCM-48** | $K_1 = 0.036$<br>$R_1 = 0.984$ | $K_2 = 14.85$<br>$R_2 = 0.993$ | $k_1 = 0.0096$<br>$K = 4.78$ | n = 0.5 |

Cytotoxicity study of MCM-48, TPA-MCM-48, CPT/TPA-MCM-48 and pure CPT was also carried out (Figure 6). MCM-48 (carrier) resulted in ≤ 35% cytotoxicity in the said doses (0.1, 0.3, 0.5 mg/ml, Figure 25). The functionalized carrier, TPA-MCM-48 showed 20.9% cytotoxicity at 0.5mg/ml dose whereas functionalized carrier loaded with drug CPT (CPT/TPA-MCM-48) recorded 58.6% cell death at 0.5mg/ml dose. These observations reveal that CPT/TPA-MCM-48 (at 0.5 mg/ml) accounted for 22% higher cytotoxicity than CPT treated group at the same dose. These results suggest that TPA-MCM-48 carrier is non-toxic to the cells. The drug loaded carrier (CPT/TPA-MCM-48) accounts for highest percentage of cytotoxicity amongst all groups implying towards improved delivery of CPT that can be of significance in cancer therapy.

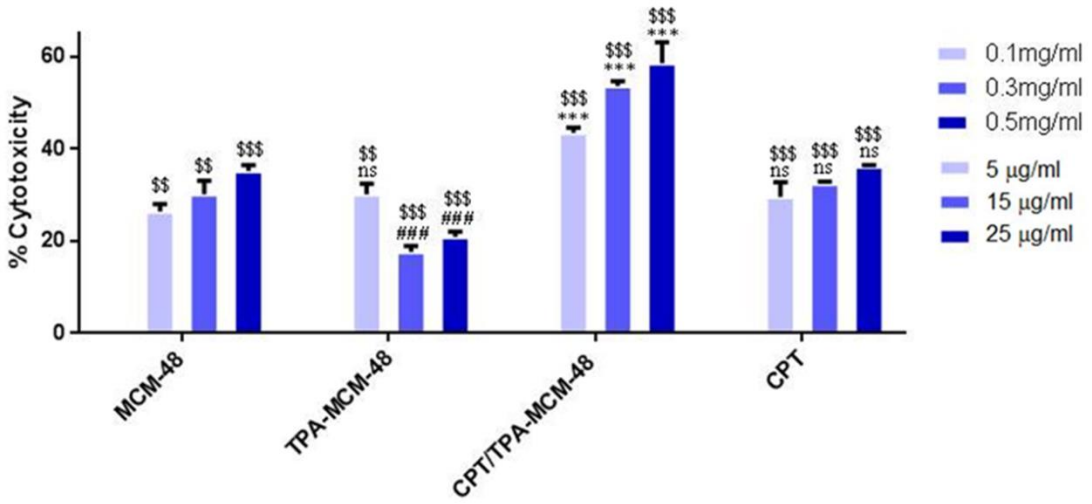



**Figure 6**. Effect of materials on the cytotoxicity of HepG2 cells. Control cells did not show cytotoxicity. Results are expressed as mean ± SD for n=3. $$P < 0.01 and $$$P < 0.001 as compared to control of respective groups and ###P < 0.001, non- significant (ns) as compared to respective concentrations of MCM-48. ***P < 0.001 as compared to respective concentrations of TPA-MCM-48.

To see the effect of geometry of carrier on released rate, release profile obtained from MCM-41 and MCM-48 has been compared (Table 13) and results shows that more delayed and slower release was observed in case of MCM-41 as compared to MCM-48. The obtained results are attributed to the well-massed transportation of the 3D interconnected pore system of MCM-48, which reduced the diffusion hindrance and assist drug diffusion into the medium.

**Table 13.** Comparison of release profile obtained from MCM-41 and MCM-48

| Materials | % of Drug release | | | Materials | % of Drug release | | |
|---|---|---|---|---|---|---|---|
| | Initial | After 10h | 30 h | | Initial | After 10h | 30 h |
| **Asp/MCM-41** | 41 | 61 | 97 | **Asp/TPA-MCM-41** | 25 | 56 | 89 |
| **Asp/MCM-48** | 43 | 80 | 97 | **Asp/TPA-MCM-48** | 38 | 71 | 81 |
| **Cap/MCM-41** | 44 | 83 | 96 | **Cap/TPA-MCM-41** | 34 | 67 | 89 |
| **Cap/MCM-48** | 46 | 94 | 98 | **Cap/TPA-MCM-48** | 33 | 65 | 93 |
| **CPT/MCM-41** | 28 | 72 | 98 | **CPT/TPA-MCM-41** | 21 | 62 | 90 |
| **CPT/MCM-48** | 36 | 74 | 98 | **CPT/TPA-MCM-48** | 30 | 68 | 97 |

Further, comparison of cytotoxic study of CPT/MCM-41, CPT/TPA-MCM-41, CPT/MCM-48 and CPT/TPA-MCM-48 also shows that CPT/TPA-MCM-48 is better systems. It shows higher toxic effect as compared to rest of the systems. Hence, CPT/TPA-MCM-48 was selected for in-vivo study.

In vivo study shows that the maximum CPT level was reached at 6 and 8 hrs after oral administration of CPT/MCM-48 and CPT/TPA-MCM-48 respectively and then decreased over the next 12 h, which indicated the prolonged residence time of the released drug in the colon with slow leaching of the drug to systemic circulation due to low permeability and compromised surface area. The difference in maximum CPT level for CPT/MCM-48 and CPT/TPA-MCM-48 may be because TPA holds the CPT molecule for longer period of time in later case which leads to slower release. The pharmacokinetic profiles shows that AUC of CPT/MCM-48 and CPT/TPA-MCM-48 in rats were 9802.13 ± 30.2 and 1358.52±30.2 ng/Lh (Table 14), which was significantly improved as compared to that of pure CPT (187.80±58 ng/Lh).

**Table 14.** Pharmacokinetic Parameters for Camptothecin delivered orally using MCM-48 and TPA-MCM-48 as carrier

| Parameters | Pure CPT (R) | CPT/MCM-48 | CPT/TPA-MCM-48 |
|---|---|---|---|
| $C_{max}$ (ng/mL) | 135.1 ± 12.5 | 1100.12 ± 5 | 1423.88 ± 5 |
| $T_{max}$ (h) | 0.5 ±1 | 6 ± 0.5 | 8 ± 0.5 |
| $t_{1/2}$ (h) | 0.8 ± 0.0 | 6.01 ± 0.2 | 4.82 ± 0.3 |
| $AUC_{0\rightarrow t}$ (ng/Lh) | 187.8 ± 58.2 | 9802.13 ± 30.2 | 13582.3 ± 30.2 |

**Annexure** describes the synthesis of MCM-41 type mesoporous silica nanoparticles (Np-MCM-41), its functionalization by TPA and their application as carrier for L-Arginine, Aspirin and Captopril. Annexure is divided into two part.

**Annexure Part-A** describes synthesis of Np-MCM-41, its functionalization (TPA-Np-MCM-41) and loading as well as characterization. Further, it describes in vitro release of L-Arginine under different condition as well as kinetic and mechanism of release.

10-30% of L-arginine was loaded into Np-MCM-41 (L-$arg_1$/Np-MCM-41, L-$arg_2$/Np-MCM-41 and L-$arg_3$/Np-MCM-41) by wet impregnation method. In vitro release study shows that more controlled release was obtained for L-$arg_1$/Np-MCM-41 compared to other systems and this is further supported by first order release kinetic model and Higuchi model (Table 15).

**Table 15.** Kinetic parameters obtained from First order release kinetic model and Higuchi model

| Model | L-arg$_1$/Np-MCM-41 | L-arg$_2$/TPA-MCM-41 | L-arg$_3$/Np-MCM-41 |
|---|---|---|---|
| **First order release kinetic model** | $K_1$ = 0.015<br>$R_1$ = 0.946 | $K_1$ = 0.0099<br>$R_1$ = 0.878 | $K_1$ = 0.0205<br>$R_1$ = 0.746 |
| **Higuchi model** | $K_2$ = 7.757<br>$R_2$ = 0.952 | $K_2$ = 4.795<br>$R_2$ = 0.932 | $K_2$ = 6.84<br>$R_2$ = 0.768 |

In vitro release of L-arg/TPA-MCM-41 was also carried out under different conditions and obtained results are shown in Table 16. The study shows that under static condition slower diffusion of L-Arginine was observed.

**Table 16.** In vitro release of L-Arginine from Np-TPA-MCM-41

| Materials | Conditions | %Release | | |
|---|---|---|---|---|
| | | Initial | After 10h | 30 h |
| **L-arg/TPA-Np-MCM-41** | stirring | 45 | 58 | 80 |
| | Static | 28 | 43 | 50 |
| | SBF (pH 7.4) | 45 | 58 | 80 |
| | SGF (pH 1.2) | 24 | 26 | 28 |

To find the release kinetic and mechanism of L-Arginine release from TPA-Np-MCM-41, release data were fitted to First order release kinetic model ($R^2$ = 0.919) and Higuchi model ($R^2$ = 0.940). The obtained results suggest that release of L-arginine follows both model with Fickian diffusion mechanism.

**Annexure Part-B** describes the loading, characterizations in vitro release, kinetics as well as mechanism of Aspirin/Captopril from Np-MCM-41 and TPA-Np-MCM-41. In vitro release study was carried out under different conditions and obtained results are shown in Table 17. The obtained results confirm that under static condition slower release was obtained for both drugs (Table 17). Further, it shows faster release of drugs under acidic pH. This may be due that protonation of C=O group of both drugs were occurred at pH 1.2 and hence is no more present for hydrogen bonding.

**Table 17.** In vitro release profile of Aspirin/Captopril from Np-MCM-41 and TPA-Np-MCM-41

| Materials | Conditions | % Release | | |
|---|---|---|---|---|
| | | Initial | After 10h | 30 h |
| **Asp/Np-MCM-41** | stirring | 41 | 91 | 97 up to 20 h |
| | Static | 18 | 22 | 23 up to 22 h |
| **Asp/TPA-Np-MCM-41** | stirring | 28 | 75 | 92 up to 25 h |
| | Static | 16 | 23 | 27 up to 25 h |
| **Asp/Np-MCM-41** | SBF (pH 7.4) | 41 | 91 | 97 up to 20 h |
| | SGF (pH 1.2) | 48 | 98 | - |
| **Asp/TPA-Np-MCM-41** | SBF (pH 7.4) | 28 | 75 | 92 up to 25 h |
| | SGF (pH 1.2) | 38 | 91 | 98 up to 18 h |
| **Cap/Np-MCM-41** | stirring | 49 | 64 | 97 up to 26 h |
| | Static | 29 | 44 | 68 |
| **Cap/TPA-Np-MCM-41** | stirring | 25 | 59 | 88 up to 26 h |
| | Static | 20 | 46 | 59 |
| **Cap/Np-MCM-41** | SBF (pH 7.4) | 49 | 64 | 97 up to 26 h |
| | SGF (pH 1.2) | 35 | 55 | 98 |
| **Cap/TPA-Np-MCM-41** | SBF (pH 7.4) | 25 | 59 | 88 up to 26 h |
| | SGF (pH 1.2) | 38 | 76 | 96 up to22 h |

Further, to see the effect of TPA on release rate, release profiles obtained from Np-MCM-41 and TPA-Np-MCM-41 has been compared (Table 17) and result shows that later systems shows more ordered and slower release rate.

To investigate the drug release kinetic and mechanism, release data were fitted to First order release kinetic model and Higuchi model and kinetic parameters are shown in Table 18.

**Table 18.** Kinetic parameters obtained from First order release kinetic model and Higuchi model

| Materials | Model | |
|---|---|---|
| | First order release kinetic model | Higuchi model |
| **Asp/Np-MCM-41** | $K_1 = 0.087$<br>$R_1 = 0.954$ | $K_1 = 19.38$<br>$R_1 = 0.949$ |
| **Asp/TPA-Np-MCM-41** | $K_2 = 0.045$<br>$R_2 = 0.992$ | $K_2 = 17.26$<br>$R_2 = 0.979$ |
| **Cap/Np-MCM-41** | $K_1 = 0.015$<br>$R_1 = 0.969$ | $K_1 = 5.47$<br>$R_1 = 0.894$ |
| **Cap/TPA-Np-MCM-41** | $K_2 = 0.026$<br>$R_2 = 0.981$ | $K_2 = 12.48$<br>$R_2 = 0.991$ |

Further, comparison of loading effeciency (LE) of MCM-41 and Np-MCM-41 was carried out and results are shown in Table 19.. Higher LE was obtained for Np-MCM-41 as compared to MCM-41 as former has higher specific surface area (1100 $m^2/g$) as compare to later one (890 $m^2/g$).

**Table 19.** Comparision of loading effecincy of MCM-41 and Np-MCM-41

| Drugs | Amount of drug mg/g of materials | |
|---|---|---|
| | MCM-41 | Np-MCM-41 |
| **Aspirin** | 52 ± 2 | 58± 2 |
| **Captopril** | 52 ± 2 | 67 ± 2 |

**Main Conclusions**

- Synthesis, functionalization and characterization MCM-41 as well as MCM-48 by various physicochemical techniques have been carried out successfully.
- Based on in vitro release study and as well as kinetic and mechanistic study, we propose that soaking method is better as compared to wet impregnation for obtaining desire release rate.
- Further, the comparison of release profiles obtained from MCM-41 and MCM-48 suggests that carrier geometry has pronounced influence on release rate of amino acids/drugs.
- It also shows that stirring as well pH of the medium have effect on release rate depending on type of interaction present between amino acid/drug and carrier.
- Modification of the carrier using TPA slows down the release rate compared to pure one and this can increase the bioavailability of drug. This is further supported by in vivo release study of CPT/MCM-48 and CPT/TPA-MCM-48.
- Kinetic and mechanistic study shows that release of all amino acids/drug follows first order kinetic and Fickian diffusion mechanism.
- MTT study confirms that functionalization of carrier by TPA does not induce toxicity into the carrier.
- Based on MTT study, CPT/MCM-48 as well as CPT/TPA-MCM-48 was selected for in-Vivo study and obtained results show that the AUC of for both systems in rats were 9802.13 ± 30.2 and 1358.52±30.2 ng/Lh respectively, which was significantly higher as compared to that of pure CPT (187.80±58 ng/Lh).
- Comparison of MCM-41 and Np-MCM-41 as carrier shows similarity in the nature of release profiles obtained from both carriers. However, for latter case, higher loading of drug is observed.

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