CHAPTER 6

In vitro controlled release study of drug (Captopril, Aspirin and Camptothecin) from MCM-48 and TPA-MCM-48

EXPERIMENTAL

In vitro controlled release of Captopril

In vitro release of Captopril was obtained by same method as described in chapter 3. In vitro release was also carried out in static condition as well as under acidic condition (pH 1.2).

Results and discussion

(i) Comparison with physical mixture

In order to see whether the Captopril molecule only physically adsorbed on the outer surface of material or not, release profile of Captopril loaded material and physical mixture of Captopril and carrier was compared and shown in Figure 1. It is clear from the Figure 1 that physical mixture shows 100% dissolution of Captopril within 1 h. while Captopril loaded material shows controlled and ordered release which suggests the presence of drug molecule inside the channel of carrier.

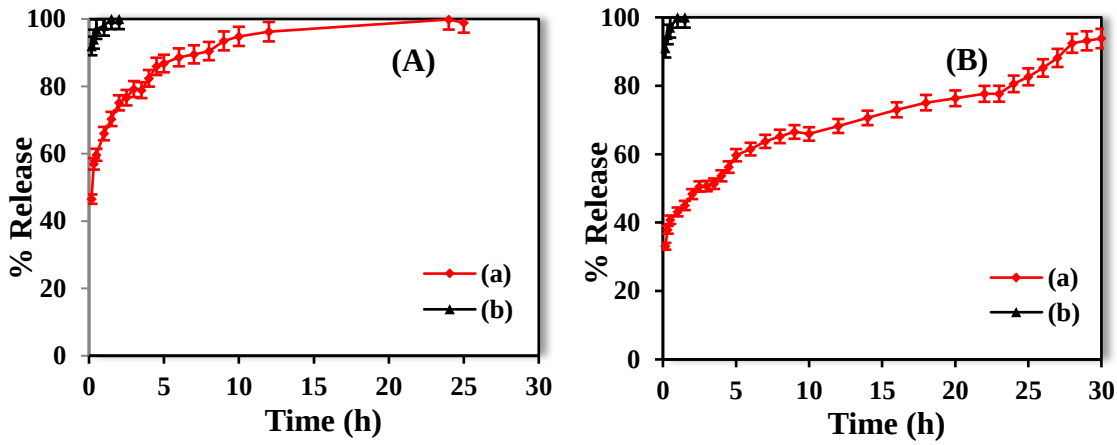

Figure 1. Comparison of release profile of (A) **a** Cap/MCM-48 and (B) **a** Cap/TPA-MCM-48 with **b** physical mixture

(ii) Effect of stirring on release rate of Captopril

To see the effect of stirring on release rate of Captopril, in vitro release study of Cap/MCM-48 and Cap/TPA-MCM-48 was carried out under stirring as well as static and results are shown in Figure 2. Under stirring condition, initially 46% Captopril is released and reached to up to 98% in 26 h for Cap/MCM-48 while in case of Cap/TPA-MCM-48; initially 33% of drug is released and reached up to 93% in 30 h. For both systems, slower release of drug is observed, under static condition which may be due to the slower diffusion of drug molecules (Figure 2).

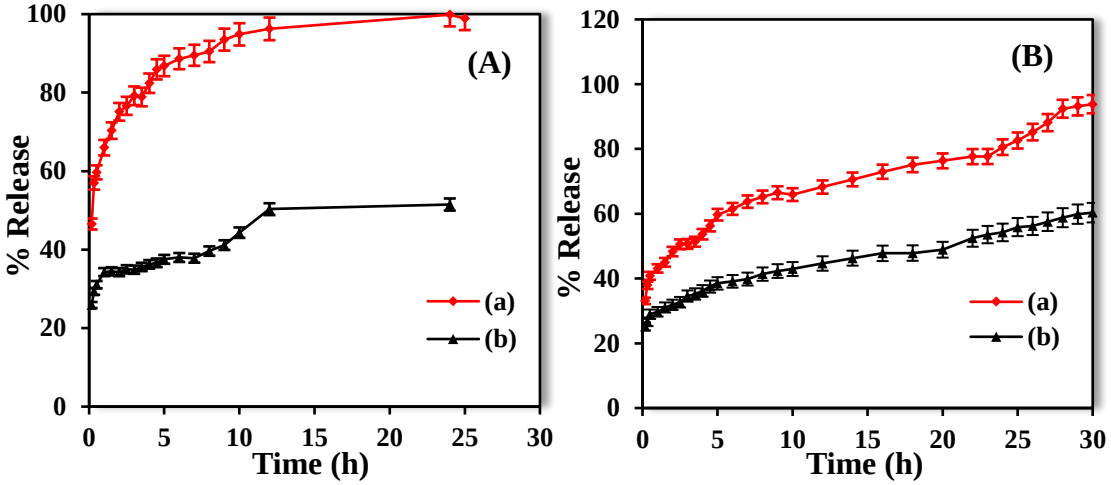

Figure 2. In vitro release profile (A) Cap/MCM-48 and (B) Cap/TPA-MCM-48 under (a) stirring and (b) static condition

(iii) Effect of pH on release rate of Captopril

To see the effect of pH on release rate of drug, in vitro release study of Captopril was carried out at different pH (1.2 and 7.4) and results are shown in Figure 3. At lower pH (SGF), higher release rate was observed for both systems (Cap/MCM-48 and Cap/TPA-MCM-48) as compared to high pH (SBF) which may be because of the action of COOH group of Captopril. However, slower release was obtained for Cap/TPA-MCM-48 compared to Cap/MCM-48. Here also TPA plays a major role in release rate of drug which was further explained in the next section.

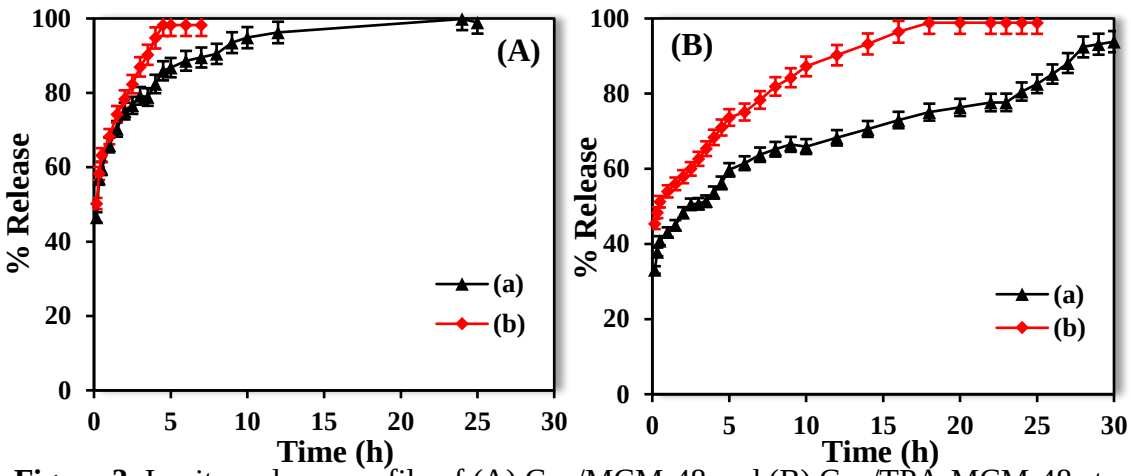

Figure 3. In vitro release profile of (A) Cap/MCM-48 and (B) Cap/TPA-MCM-48 at pH (a) 7.4 (SBF) and (b) 1.2 (SGF)

(iv) Effect of TPA on release rate of Captopril

To investigate the action of TPA on release rate of drugs, release profile of drug loaded into pure materials were compared with functionalized materials and results are shown in Figure 4. Initially, 46% and 33% of Captopril is released and reached up to 94% and 65% in 10 h from MCM-48 and TPA-MCM-48, respectively. Further, it reached up to 98% in to 25 h and 93% in 29 h for MCM-48 and TPA-MCM-48, respectively. Here, more controlled release profile is obtained from TPA-MCM-48 as compared to pure MCM-48.

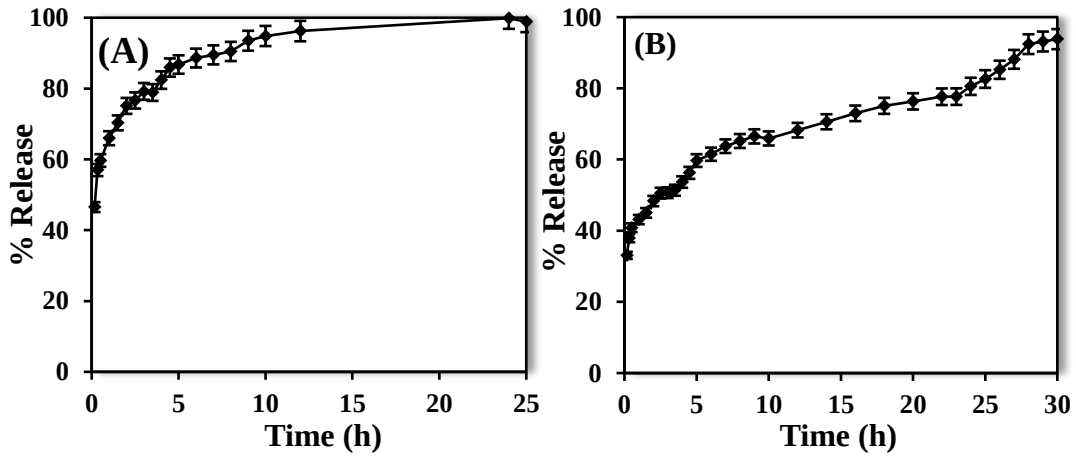

Figure 4. Comparison of release profile of (a) Cap/MCM-48 and (b) Cap/TPA-MCM-48

Burst release is observed for Cap/MCM-48 which may be due the release of drug molecules present on the surface of carrier. However, more controlled release profile is obtained from TPA-MCM-48 as compared to pure MCM-48. This may be because of the more interaction between the drug molecules and TPA-MCM-48. As stated earlier, TPA has terminal free oxygen through which it can bind with drug. This may be the reasons of slower release of drugs from TPA-MCM-48.

FTIR analysis of Cap/TPA-MCM-48 after release study was also carried out and spectrum is shown in Figure 5. FTIR spectrum of Cap/TPA-MCM-48 is similar with Figure 2b (TPA-MCM-48) which confirmed that structure of TPA remains intact in Cap/TPA-MCM-48 even after release study and it's truly act as functionalizing agent.

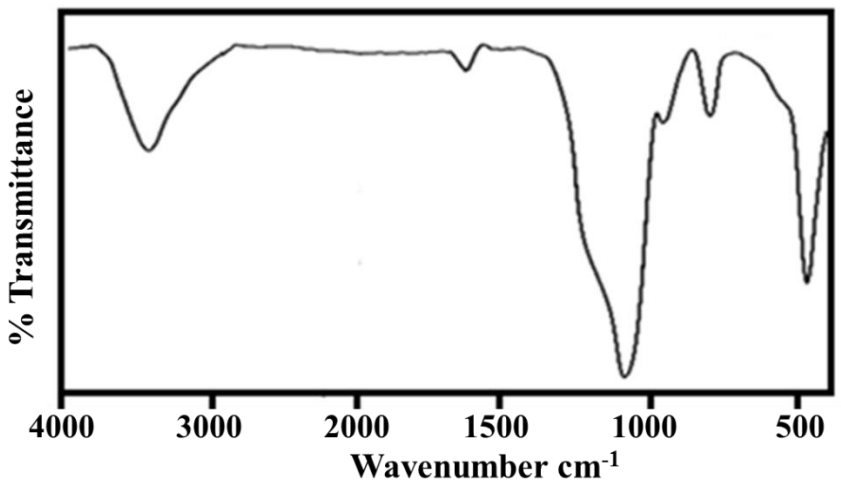

Figure 5. FTIR spectrum of Cap/TPA-MCM-48 after release study

Kinetics and Mechanism

To investigate drug release kinetic and mechanism, The Captopril release data up to 10 h were fitted with First ordered release Kinetic Model and Higuchi Model.

(i) First ordered release kinetic model

First order release kinetic model is applied to study the dissolution of drug encapsulated in porous matrices. According to this model rate of release is concentration dependent. Figure 6 shows plot of log of percentage remaining data against time and kinetic parameters are shown in Table 1. It was found that the release of Captopril follows the first order kinetic with linearity. Further, higher linearity and higher co-relation coefficient were obtained for Cap/TPA-MCM-48 (R^2 = 0.9744) as compared to Cap/MCM-48 (0.9731).

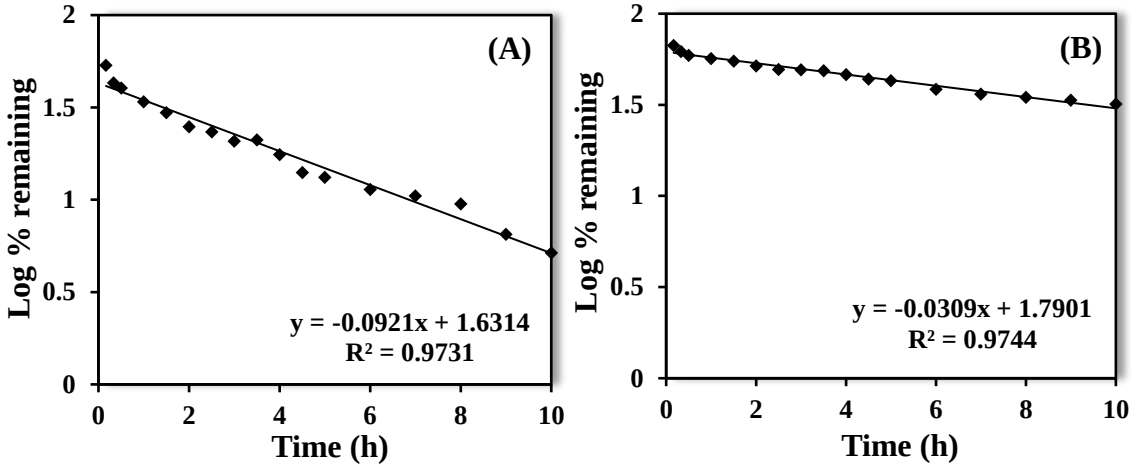

Figure 6. First order release kinetic model for (A) Cap/MCM-48 and (B) Cap/TPA-MCM-48

(ii) Higuchi Model

Figure 7 shows Higuchi model of Cap/MCM-48 and Cap/TPA-MCM-48 which describes the percentage release versus square root of time dependent process based on Fickian diffusion. According to this model release mechanism of drug involves simultaneous penetration of SBF into the pores, dissolution of drug molecule and diffusion of these molecules from the pores. The release mechanism of Captopril is best explained by this model with high linearity and high correlation coefficient (R^2) value for Cap/TPA-MCM-48 (R^2 = 0.9814) as compared to Cap/MCM-41 (R^2 = 9431). Higher value of R^2 for suggests, more ordered release of Captopril from TPA-MCM-48

as compared to pure MCM-48. Thus kinetic and mechanistic study shows that drug release is concentration dependent process, follows first order release kinetics as well as it follows the Fickian diffusion mechanism.

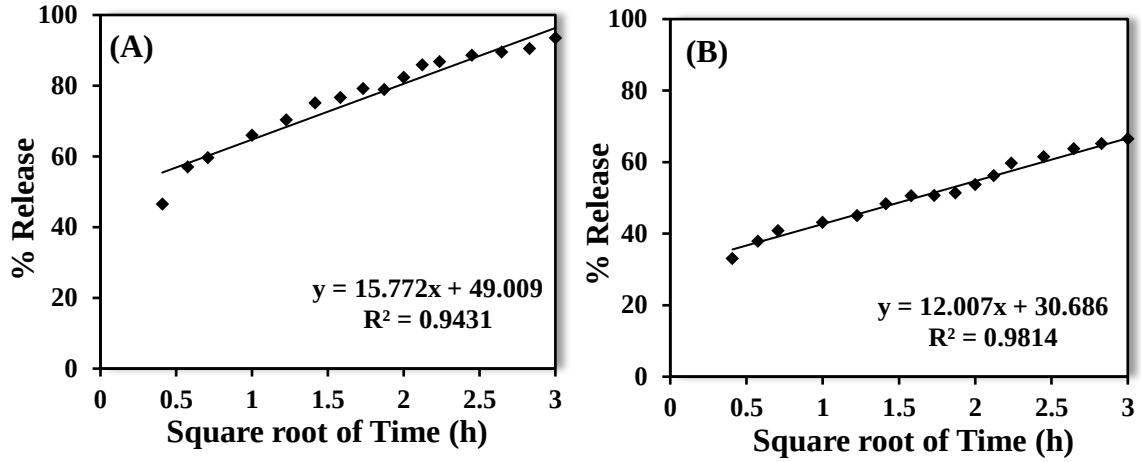

Figure 7. Higuchi Model for (A) Cap/MCM-48 and (B) Cap/TPA-MCM-48

Table 1. Kinetic parameters obtained from First order release kinetic model and Higuchi model

Model	Cap/MCM-48	Cap/TPA-MCM-48
First order release kinetic model	K_1 = 0.092 R_1 = 0.973	K_1 = 0.030 R_1 = 0.974
Higuchi model	K_2 = 15.77 R_2 = 0.943	K_2 = 12.00 R_2 = 0.981

In vitro controlled release of Aspirin

Release profile of Aspirin was also obtained by same method as described in chapter 3. To see the effect of pH on release rate of drug, release study was also carried out in SGF (pH 1.2) was also carried. For finding out, whether the drug molecules are physically adsorbed or present inside the channels of the carrier, release study was carried out in SBF for physical mixture of drug (Aspirin) and carrier, prepared by mixing and grinding them physically.

Results and Discussion

(i) Comparison with Physical mixture

In order to see whether the Aspirin molecule only physically adsorbed on the outer surface of material or not, release profile of Aspirin loaded material and physical mixture of Aspirin and carrier was compared and shown in Figure 8. It is clear from the Figure 8 that physical mixture shows 100% dissolution of Aspirin within 1 h. while Aspirin loaded material shows controlled and ordered release which suggests the presence of Aspirin molecule inside the channel of carrier**.**

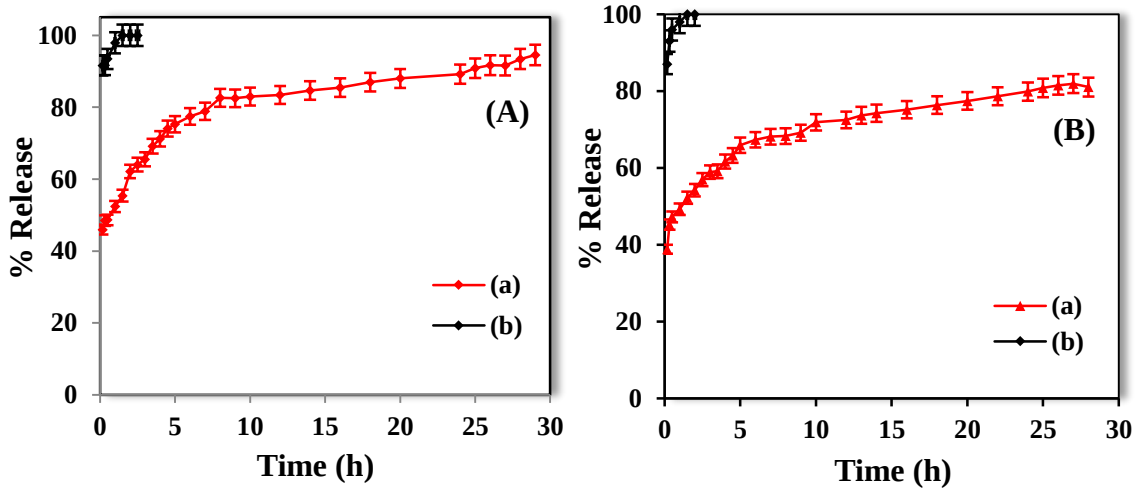

Figure 8. Comparison of release profile of (A) **a** Asp/MCM-48 and (B) **a** Asp/TPA-MCM-48 with **b** physical mixture

(ii) Effect of stirring on release rate

In order to understand the effect of stirring on release rate of drug, in vitro release study was carried out under two different conditions: (1) stirring as well as (2) static and results are shown in Figure 9. Under stirring condition, in case of Asp-MCM-48, initially 43% Aspirin is released and reached up to 97% in 30 h while in case of Asp/TPA-MCM-48 initially 38% of drug is released and reached up to 81% in 30 h. However, because of the slower diffusion of drug under static condition, slower release of Aspirin is observed for both systems (Figure 1).

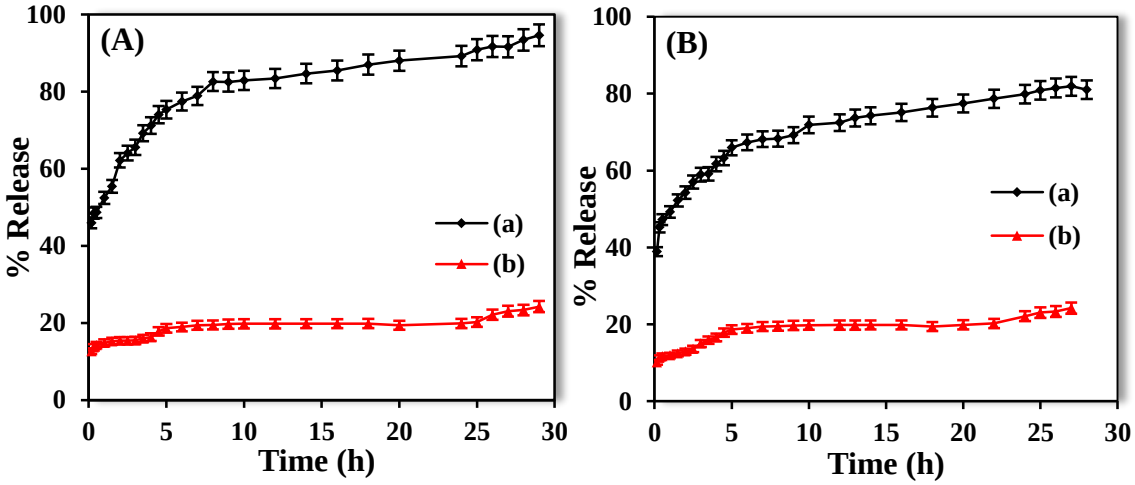

Figure 9. In vitro release profile (A) Asp/MCM-48 and (B) Asp/TPA-MCM-48 under (a) stirring and (b) static condition

(iii) Effect of pH on release rate

In order to see the effect of pH on release rate of Aspirin, in vitro release study was carried at two different pH and results are shown in Figure 10. At pH 1.2 (SBF), release rate becomes faster as compared to at pH 7.4 (SGF) for both Asp/MCM-48 and asp/TPA-MCM-48. This may be due to protonation of COOH group of Aspirin that occurred at pH 1.2 and hence C=O group is no longer present for hydrogen bonding with surface Si-OH group of materials. Thus, the interaction between Aspirin and materials become weak and hence faster release is observed under SGF.

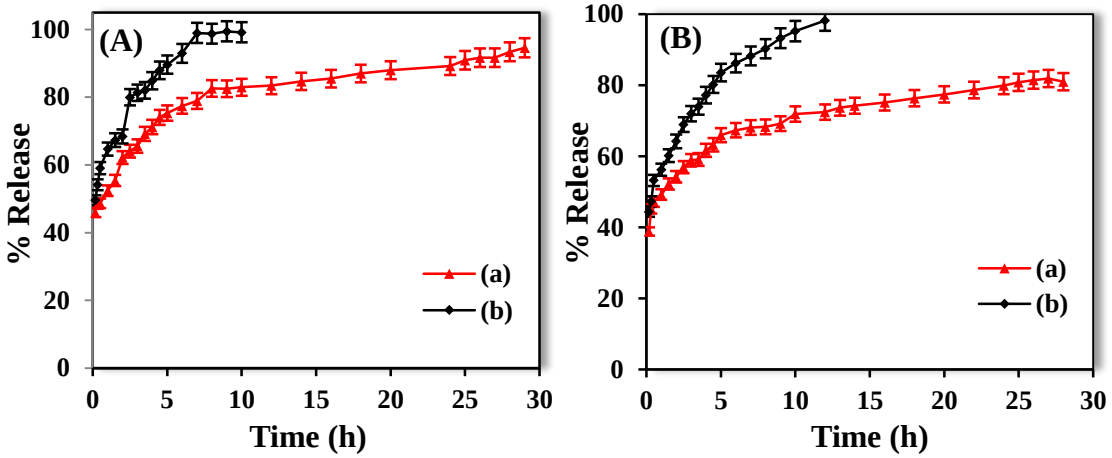

Figure 10. In vitro release profile of (A) Asp/MCM-48 and (B) Asp/TPA-MCM-48 at pH (a) 7.4 (SBF) and (b) 1.2 (SGF)

(iv) Effect of TPA on release rate

To see the effect of TPA on release rate of Aspirin, release profile of Asp/MCM-48 and Asp/TPA-MCM-48 was compared and results are shown in Figure 11. Initially, 43% and 38% of Aspirin is released and reached up to 80% and 71% in 10 h from MCM-48 and TPA-MCM-48, respectively. It reached up to 97% and 81% in 30 h for MCM-48 and TPA-MCM-48, respectively. Here, more controlled release profile is obtained for Asp/TPA-MCM-48 as compared to Asp/MCM-48.

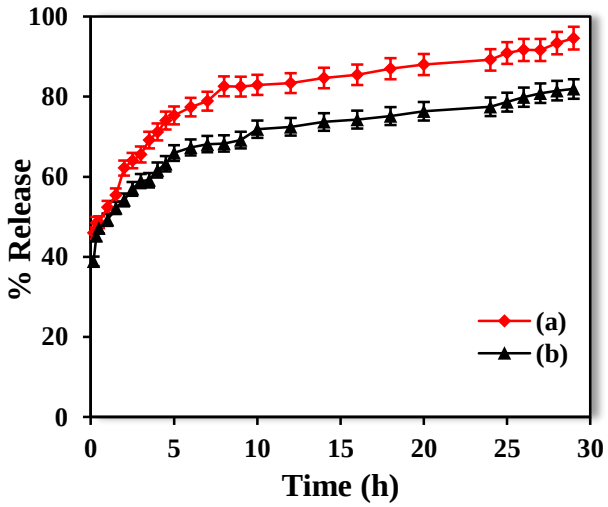

Figure 11. Comparison of release profile of (a) Asp/MCM-48 and (b) Asp/TPA-MCM-48

This is may be because of more attractive interaction between the Aspirin molecules and TPA-MCM-48. As stated earlier, TPA has terminal free oxygen through which it can bind with drug. This may be the reasons of slower release of Aspirin from TPA-MCM-48.

Further to confirm that TPA act as only functionalizing agent and its structure remain intact during release study, FTIR analysis of Asp/TPA-MCM-48 after release study was also carried out and spectrum is shown in Figure 12. FTIR spectrum of Asp/TPA-MCM-41 is similar with Figure 2b (TPA-MCM-48) which confirmed that structure of TPA remains intact in Asp/TPA-MCM-48 even after release study and it's truly act as functionalizing agent.

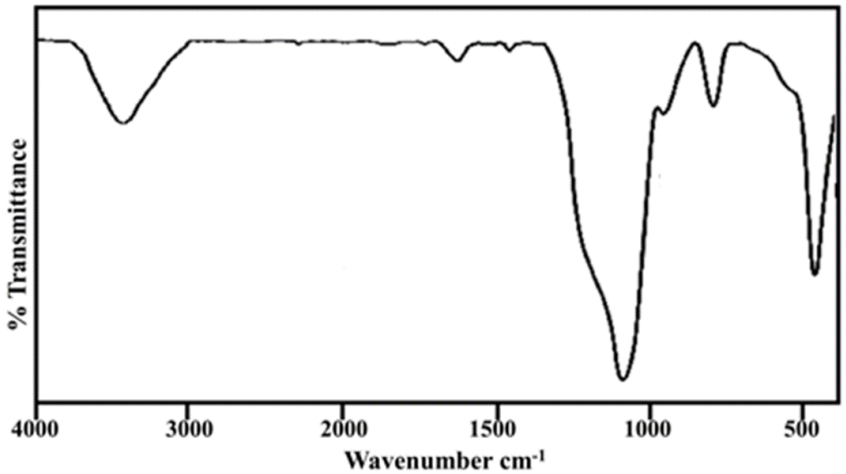

Figure 12. FTIR spectra of Asp/TPA-MCM-48 after release study

Kinetics and mechanism

To study the drug release kinetic and mechanism, the Aspirin release data up to 10 h were fitted with First ordered release Kinetic Model, Higuchi Model. The type of diffusion of Aspirin in support as well as the relation between the surface properties and release rate was studied by Korsmeyer-Peppas Model (KPM) and Extended Kinetic Model (EKM).

(i) First order release kinetic model

First order release kinetic model is used to study the dissolution of drug encapsulated in porous matrices. According to this model, rate of release is concentration dependent. Figure 13 shows first ordered release kinetic model of Asp/MCM-48 as well as Asp/TPA-MCM-48, where log of % remaining data are plotted against time in h. The First order release kinetic model was best fitted with higher linearity and higher co-relation coefficient (R^2= 0.9707) for Asp/TPA-MCM-48. This suggests the, Aspirin release is concentration dependent process and more ordered release is obtained for Asp/TPA-MCM-48 system.

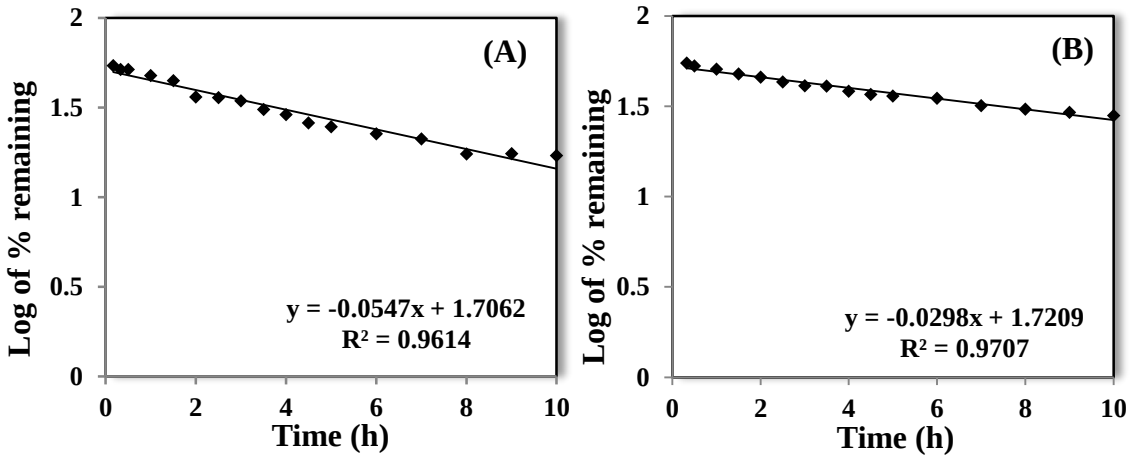

Figure 13. First order release kinetic model of (A) Asp/MCM-48 and (B) Asp/TPA-MCM-48

(ii) Higuchi model

The Higuchi model (Figure 14) describes the percentage release versus square root of time dependent process based on Fickian diffusion. According to this model release mechanism of drug involves simultaneous penetration of SBF into the pores, dissolution of drug molecule and diffusion of these molecules from the pores. The release mechanism of Aspirin is best explained by this model with high linearity and high correlation coefficient (R^2 = 0.9838) for Asp/TPA-MCM-48. This suggests that release of Aspirin follows Fickian diffusion mechanism as well as more ordered release profile was obtained in case of Asp/TPA-MCM-48.

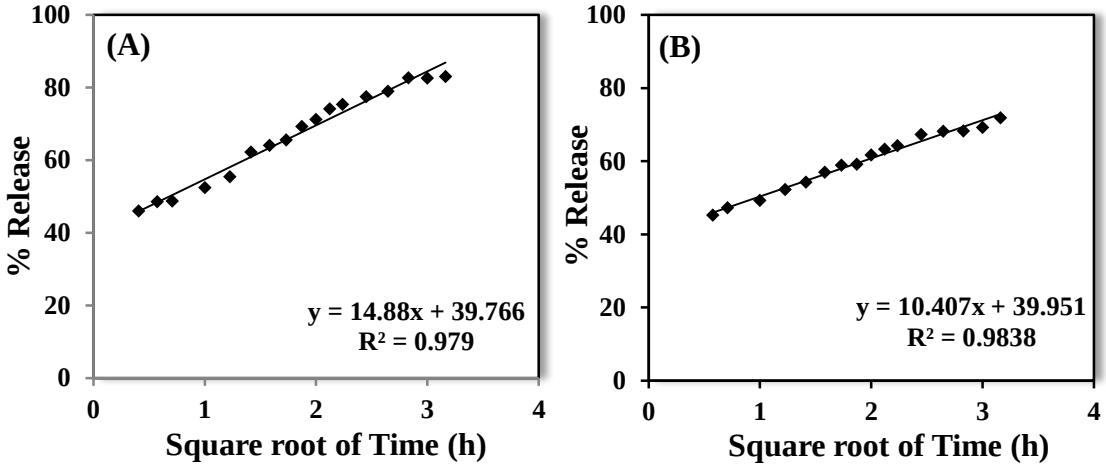

Figure 14. Higuchi Model for (A) Asp/MCM-48 and (B) Asp/TPA-MCM-48

(iii) Korsmeyer-Peppas Model

Korsmeyer and Peppas (1984) developed an empirical equation to analyze both Fickian and non-Fickian release of drug. $M_t/M_\infty = Kt^n$. n is the empiric exponent which indicates type of release mechanism, where n = 0, 1.0 and 0.5 indicates Zero-Order, First-order and Higuchi model respectively. In the present case, the found value of n is 0.48 and 0.5 for Asp/MCM-48 and Asp/TPA-MCM-48 respectively, confirming that the present systems follow Higuchi model. For KPM, higher linearity and correlation Co-efficient (R^2) was obtained for Asp/TPA-MCM-48 (Figure 15).

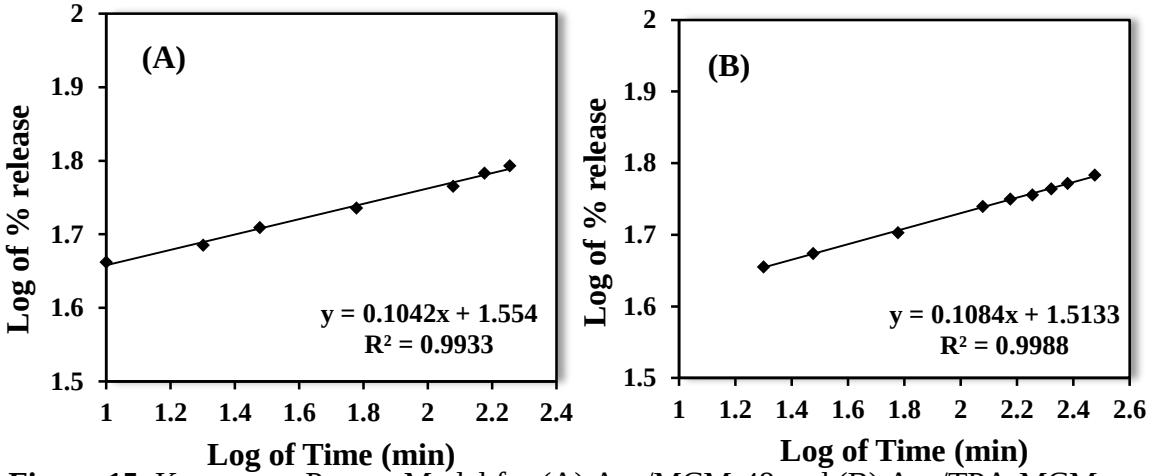

Figure 15. Korsmeyer Peppas Model for (A) Asp/MCM-48 and (B) Asp/TPA-MCM-48

(iv) Extended Kinetic Model

EK Model described the drug concentration dynamics in the bulk liquid, on solid and at the interface. It includes the diffusion steps for the drug transport in pores and in the external liquid film (surrounding the carrier) to the bulk liquid. Due to the concentration gradients, the drug follows diffusion path in the pores and external liquid film. The estimated kinetic parameters of EKM are shown in Table 2 and EKM prediction of the drug concentration dynamics in the bulk liquid, on solid and at the interface are displayed in Figure 16 for Asp/MCM-48 and Asp/TPA-MCM-48. From Figure 16, it is clear that more ordered and delayed release was obtained for Asp/TPA-MCM-41. Concentration of drug was higher in bulk liquid for Asp/MCM-48 compared to Asp/TPA-MCM-48. On comparing the estimated desorption-adsorption equilibrium constants $K = k_2/k_1$ from Table 2, it is clear that release tendency is higher for Asp/MCM-48 ($K = 9.0\times10^{-2}$) compared to Asp/TPA-MCM-48 ($K = 6.3\times10^{-2}$) and hence controlled release profile was obtained for latter case. This may be because of the interaction of Aspirin with TPA which can control the rate of release for longer time.

Concentration Gradient

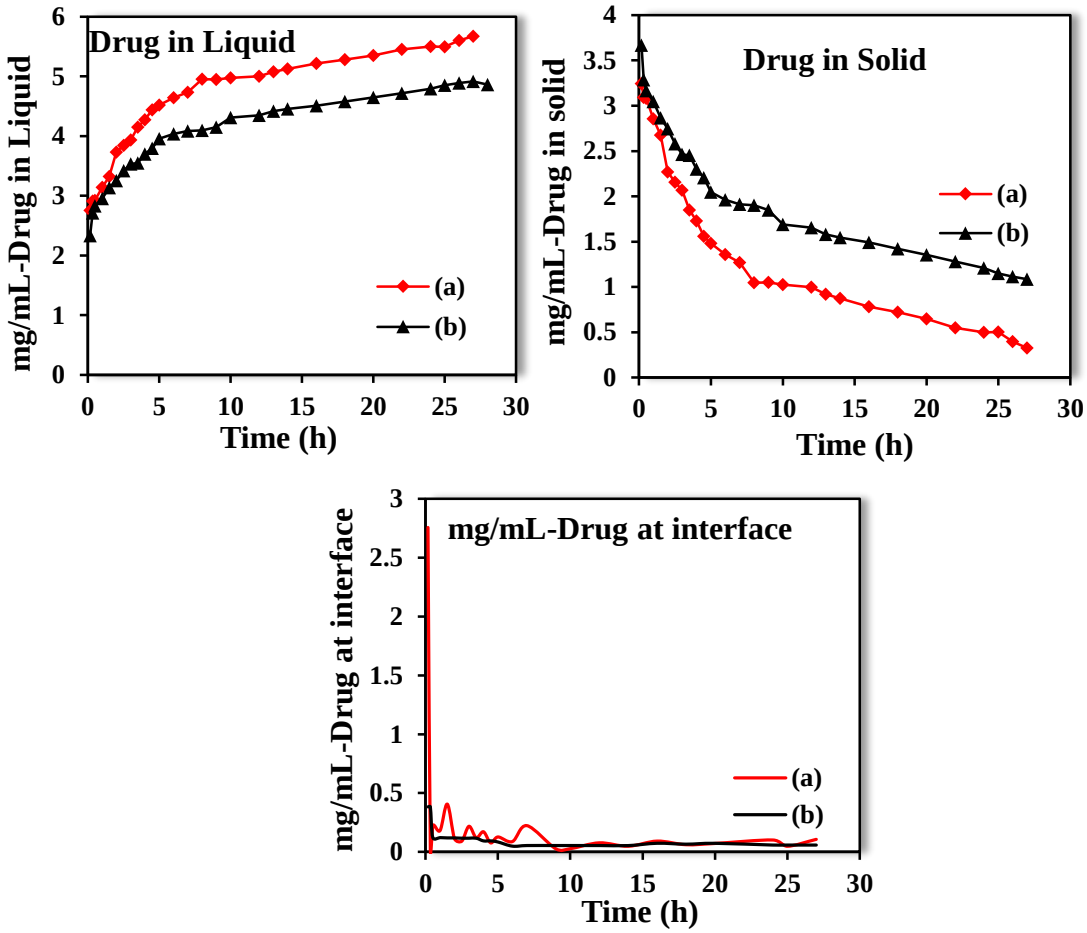

Figure 16. Experimental data and predictions of Aspirin release from MCM-48 and TPA-MCM-48 in SBF by Extended Kinetic Model

Table 2. Estimated parameters of KPM and EKM model for Aspirin 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	
	Extended Kinetic Model (EKM)	Korsmeyer-Peppas Model (KPM)
Asp/MCM-48	k_1= 0.0044 K= 9.0* 10^{-2}	n = 0.48
Asp/TPA-MCM-48	k_1= 0.084 K= 6.3* 10^{-2}	n = 0.5

In vitro controlled release of Camptothecin

In vitro release of CPT was carried out by soaking drug loaded samples in 100 mL of SBF at 200 rpm at 37 ^{o}C temperature under stirring as well as static condition. At proper time interval, 1 mL of release fluid was taken and fresh SBF was added to maintain the constant volume of the system. The 1 mL fraction was diluted and analyzed for CPT contains using UV-Visible spectrophotometry at 370 nm. All the experiments were repeated three times. For comparison of both systems, the same concentration of drug was taken for release study.

Results and Discussion

(i) Comparison with Physical mixture

In order to see whether the CPT molecule only physically adsorbed on the outer surface of material or not, release profile of CPT loaded material and physical mixture of CPT and carrier was compared and shown in Figure 17. It is clear from the Figure 17 that physical mixture shows 100% dissolution of CPT within 1 h. while CPT loaded material shows controlled and ordered release which suggests the presence of CPT molecule inside the channel of carrier**.**

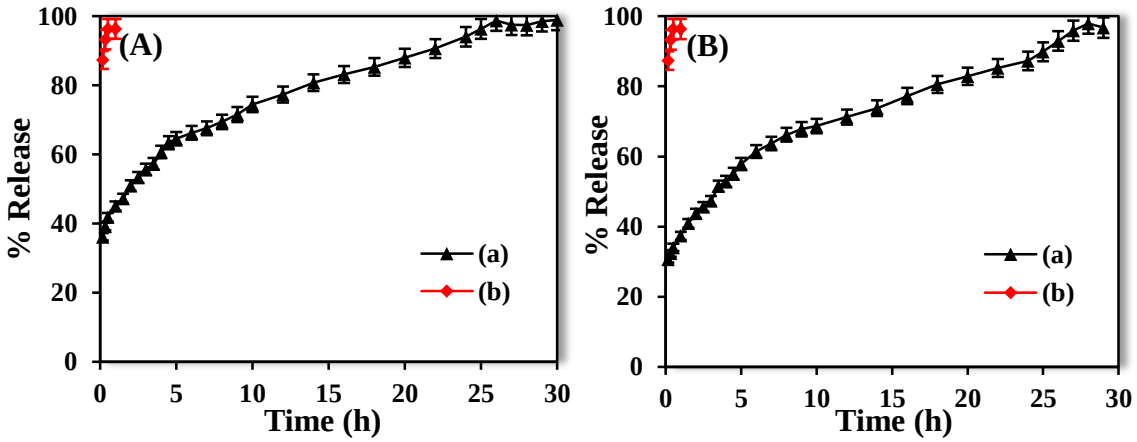

Figure 17. Comparison of release profile of (A) **a** CPT/MCM-48 and (B) **a** CPT/TPA-MCM-48 with **b** physical mixture

(ii) Effect of Stirring

To evaluate the effect of stirring on release rate of CPT, release study was carried out under stirring and static condition and results are shown in Figure 18. It is observed that under static condition slower release profile is obtained for both systems, CPT/MCM-48 and CPT/TPA-MCM-48. Under static condition, initially, 27% and 18% of CPT is release which is reached up to 42% and 38% in 10h from MCM-48 and TPA-MCM-48, respectively. However, comparatively fast release profile is obtained under stirring condition for both systems. Slower diffusion of CPT molecules, under static condition could be the reason of slower release of CPT.

To see the effect of pH on release rate, release study was also carried out in SGF which is similar to that of result which was obtained in SBF.

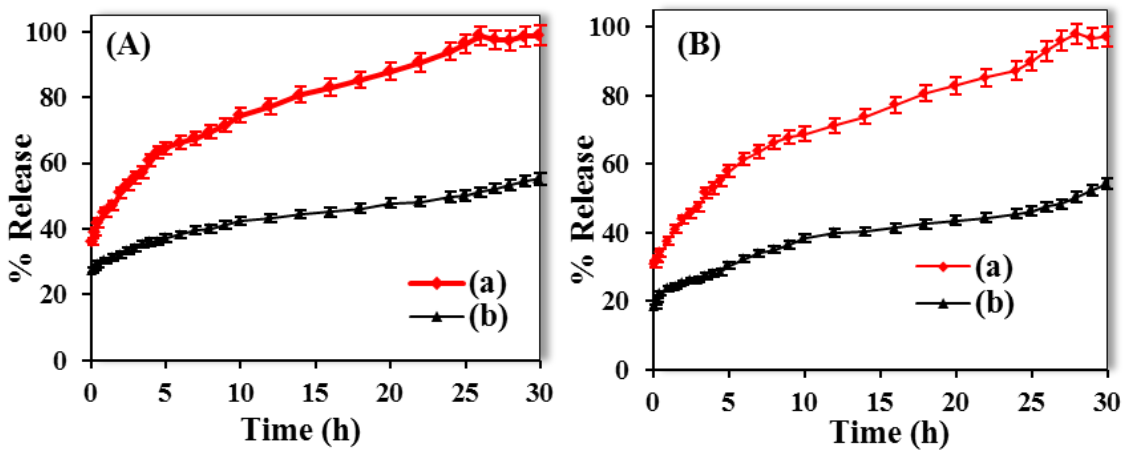

Figure 18. In vitro release profile of (A) CPT/MCM-48 and (B) CPT/TPA-MCM-48 under (a) stirring and (b) static condition

(iii) Effect of TPA on release rate

To see the influence of TPA on release rate of CPT, release profile of CPT/MCM-48 and CPT/TPA-MCM-48 was compared and results are shown in Figure 19. Initially, 36% and 30% of CPT is released and reached up to 74% and 68% in 10 h from MCM-48 and TPA-MCM-48, respectively. It further reached up to 98% and 97% in 30 h for MCM-48 and TPA-MCM-48, respectively. Here, slower release profile is obtained for CPT/TPA-MCM-48 as compared to CPT/MCM-48. This is may be because of more attractive interaction between the CPT molecules and TPA-MCM-48. As stated earlier, TPA has terminal free oxygen through which it can bind with drug. This is may be the reasons of slower release of CPT from TPA-MCM-48.

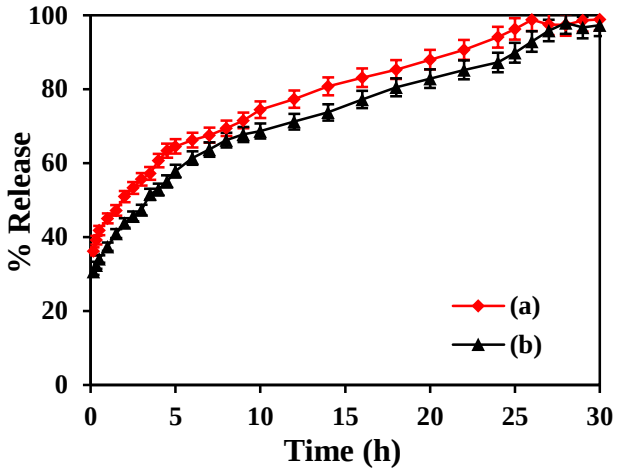

Figure 19. Comparison of release profile of CPT/MCM-48 and CPT/TPA-MCM-48

FTIR analysis of CPT/TPA-MCM-48 after release study was also carried out and spectrum is shown in Figure 20. FTIR spectrum of CPT/TPA-MCM-48 is similar with Figure 2b (TPA-MCM-48) which confirmed that structure of TPA remains intact in CPT/TPA-MCM-48 even after release study and it's truly act as functionalizing agent.

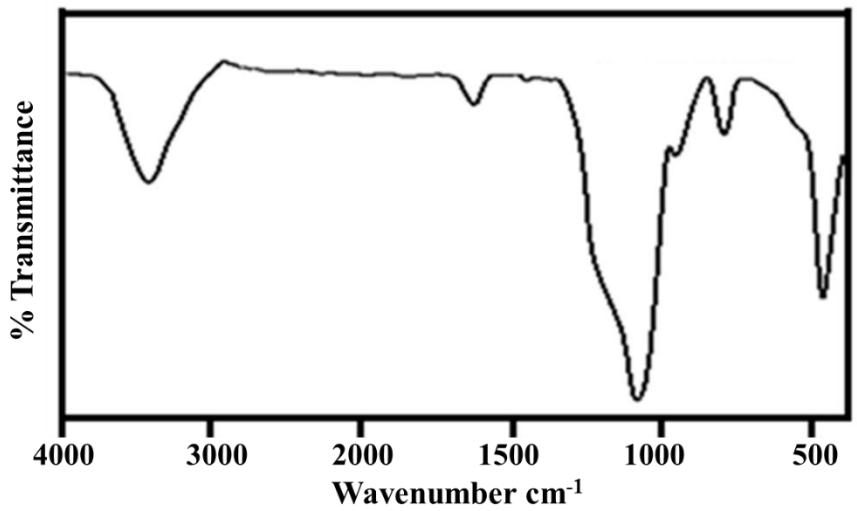

Figure 20. FTIR spectrum of CPT/TPA-MCM-48 after release study

Kinetics and Mechanism

To investigate drug release kinetic and mechanism, The CPT release data were fitted with First ordered release Kinetic Model, Higuchi Model. The type of diffusion of CPT in support as well as the relation between the surface properties and release rate was studied by Korsmeyer-Peppas Model (KPM) and Extended Kinetic Model (EKM).

(i) First order release kinetic model

First order release kinetic model is used to study the dissolution of drug encapsulated in porous matrices. According to this model, rate of release is concentration dependent. Figure 21 shows first ordered release kinetic model of CPT/MCM-48 and CPT/TPA-MCM-48, where log of % remaining data are plotted against time in hr. The First order release kinetic model was best fitted with higher linearity and higher co-relation coefficient (R^2 = 0.9841) for CPT/TPA-MCM-48. This suggests the, CPT release is concentration dependent process and more ordered release is obtained for CPT/TPA-MCM-48 system.

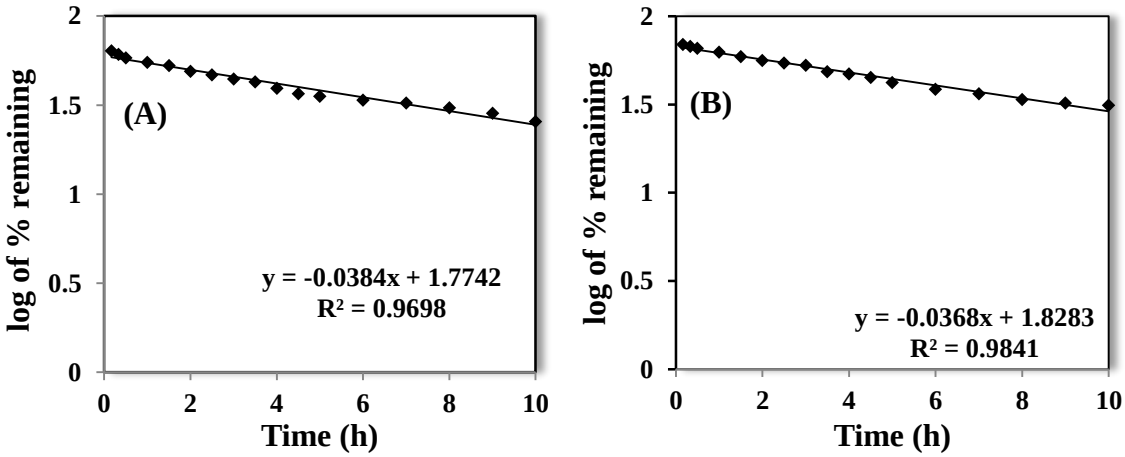

Figure 21. First order release kinetic model of (A) CPT/MCM-48 and (B) CPT/TPA-MCM-48

(ii) Higuchi model

The Higuchi model (Figure 22) describes the percentage release versus square root of time dependent process based on Fickian diffusion. According to this model release mechanism of drug involves simultaneous penetration of SBF into the pores, dissolution of drug molecule and diffusion of these molecules from the pores.

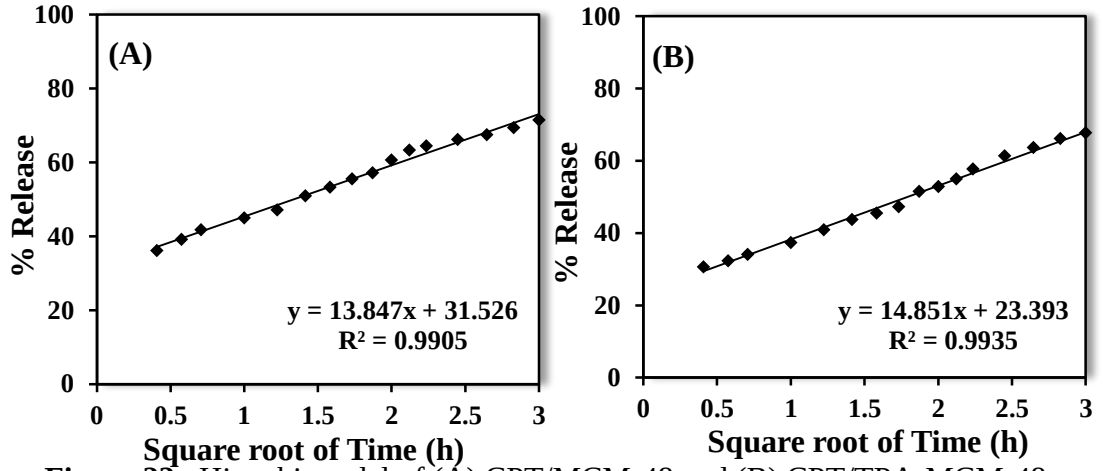

Figure 22. Higuchi model of (A) CPT/MCM-48 and (B) CPT/TPA-MCM-48

The release mechanism of CPT is best explained by this model with high linearity and high correlation coefficient (R^2 = 0.9935) for CPT/TPA-MCM-48.This suggests that release of CPT follows fickian diffusion mechanism as well as more ordered release profile was obtained in case of CPT/TPA-MCM-48.

(iii) Korsmeyer-Peppas Model

Korsmeyer and Peppas (1984) developed an empirical equation to analyze both Fickian and non-Fickian release of drug. Mt/M∞ = Ktn. n is the empiric exponent which indicates type of release mechanism, where n = 0, 1.0 and 0.5 indicates Zero-Order, First-order and Higuchi model respectively. In the present case, the found value of n is 0.49 and 0.5 for CPT/MCM-48 and CPT/TPA-MCM-48 respectively (Figure 23), confirming that the present systems follow Higuchi model. For KPM, higher linearity and correlation Co-efficient (R^2) was obtained for CPT/TPA-MCM-48.

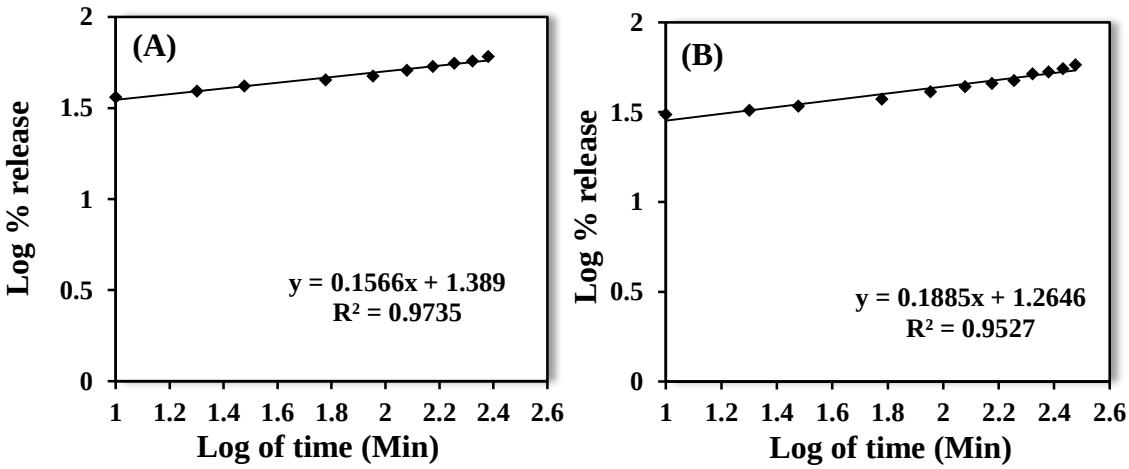

Figure 23. KPM model for (A) CPT/MCM-48 and (B) CPT/TPA-MCM-48

(iv) Extended Kinetic Model

EKM model described the drug concentration dynamics in the bulk liquid, on solid and at the interface. It includes the diffusion steps for the drug transport in pores and in the external liquid film (surrounding the carrier) to the bulk liquid. Due to the concentration gradients, the drug follows diffusion path in the pores and external liquid film. The estimated kinetic parameters of EKM model are shown in Table 2 and EKM prediction of the drug concentration dynamics in the bulk liquid, on solid and at the interface are displayed in Figure 24 for CPT/MCM-48 and CPT/TPA-MCM-48. On comparing the estimated desorption-adsorption equilibrium constants $K = k_2/k_1$ from Table 3, it is clear that release tendency is higher for CPT/MCM-48 (K = 6.19) compared to CPT/TPA-MCM-48 (K = 4.76). This may be due to the strong interaction of CPT with terminal oxygen of TPA which can hold the CPT molecules for longer time.

Concentration Gradient

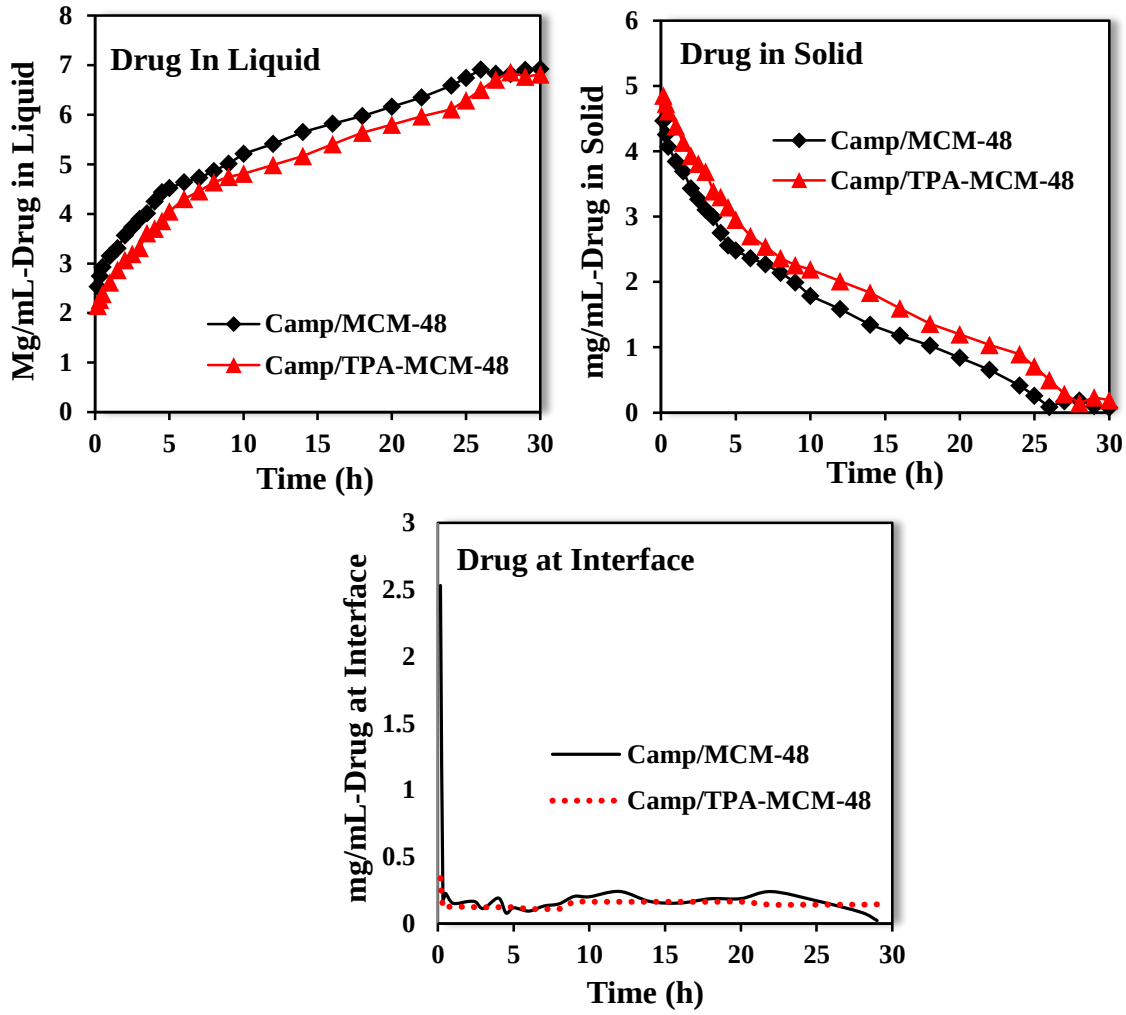

Figure 24. Experimental data and predictions of CPT release from MCM-48 and TPA-MCM-48 in SBF by Extended Kinetic Model

Table 3 Estimated parameters of KPM and EKM model for CPT 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	
	Extended Kinetic (EKM)	Korsmeyer-Peppas (KPM)
CPT/MCM-48	k_1= 0.0059 K= 5.75	n = 0.49
CPT/TPA-MCM-48	k_1= 0.0096 K= 4.78	n = 0.5

Cytotoxicity study

In Vitro Study:

Human hepatocellular carcinoma (HepG2) cells were procured from National Centre for Cell Science, Pune, India. Cells were incubated in a water jacketed CO_2 incubator (Thermo Scientific, Forma series II 3111, USA) at 37 °C with 5% CO_2 and cultured in Dulbecco's minimal eagle medium (DMEM) containing 10% Fetal Bovine Serum (FBS) and 1% antibiotic-antimycotic solution. Cells were sub-cultured every third day using trypsin.

Cytotoxicity Assay:

Cytotoxicity was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells (7×10^3 cells/well) were seeded in 96-well culture plates and incubated for 24 h. Later, cells were treated with 0.1, 0.3 and 0.5 mg/ml concentrations of MCM-48, TPA-MCM-48, CPT/TPA-MCM-48 or CPT for 24 h and untreated cells were taken as control. This was followed by addition of 100 μL of MTT (0.5 mg/ml in incomplete medium) in each well and incubated for 4 h at 37 °C. Formazan formed at the end of reaction was dissolved in 150 μL of DMSO and absorbance was read at 540 nm using Synergy HTX Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT, USA) and the percentage cytotoxicity was calculated relative to control.

Results and discussion

Evaluation of in vitro cytotoxicity-MTT assay

Topoisomerase-I inhibitors constitute an important class of the current anticancer drugs and most of Topo-I inhibitors are analogues of Camptothecin (CPT). Low bioavailability, poor water solubility and other shortcomings hinder their anticancer activity and hence a carrier can be used to improve their potential. In the present study, HepG2 cells were treated with MCM-48, TPA-MCM-48, CPT/TPA-MCM-48 or CPT and MTT assay was performed to assess the mitochondrial dysfunction and anticancer potential of test compounds. Reduction of MTT occurs in metabolically active cells and the formazan produced at the end of the assay is a measure of the viability of the cells that also reflects upon the functional status of mitochondria.

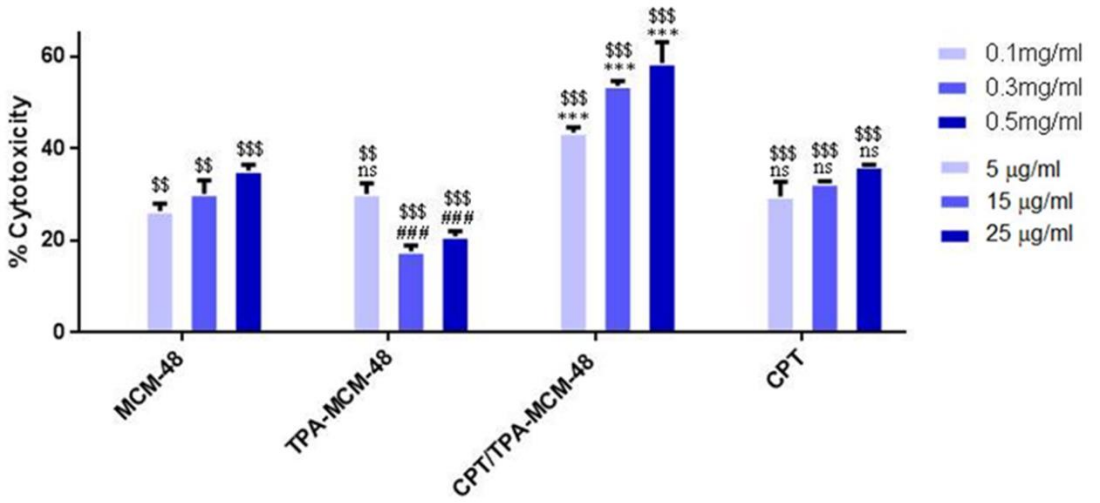

Figure 25. Effect of materials on the cytotoxicity of HepG2 cells. Control cells did not show cytotoxicity. Results are expressed as mean ± SD for n=3. $$P < 0.01 and $$$P < 0.001 as compared to control of respective groups and ###P < 0.001, non- significant (ns) as compared to respective concentrations of MCM-48. ***P < 0.001 as compared to respective concentrations of TPA-MCM-48.

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**.**

Conclusion

- In vitro release study of shows that stirring has great influence on release rate of all the drugs. Under static condition, slower release was observed for all the drugs, as in this condition diffusion of molecules became slower.
- In vitro release study under different acidic pH shows that release rate of Captopril as well as Aspirin has become faster at lower pH. At lower pH, C=O group of drug became protonated and hence interaction between drug and carrier decreases. However, pH does not show any effect on release rate of Camptothecin.
- Further, comparison of release profile of drug loaded materials and physical mixture (drug + carrier) shows that drug molecules are truly present inside the channels of carrier and not on the surface.
- It also shows that TPA shows tremendous effect on release rate of all the drugs. As having number of terminal oxygen through which it binds with drugs and hold them for longer time. Hence, slower and ordered release was obtained for Cap/TPA-MCM-48, Asp/TPA-MCM-48 and CPT/TPA-MCM-48 systems.
- FTIR after release study of Cap/TPA-MCM-48, Asp/TPA-MCM-48 and CPT/TPA-MCM-48 shows that TPA remains intact even after release and hence prove that TPA acts truly as functionalizing agent.
- Kinetic and mechanistic study shows that release of Captopril follows first ordered release kinetic model and Higuchi diffusion mechanism. Release of Aspirin as well as Camptothecin also follows the same mechanism which was further supported by Korsmeyer-Peppas Model and Extended kinetic Model.
- For behaving as true carrier, material should be non-cytotoxic. For finding this, MTT study of MCM-48, TPA-MCM-48, CPT/TPA-MCM-48 and pure CPT has also been carried out which suggests that all materials are non-cytotoxic accept one which are drug loaded.

Effect of carrier on drug release

Release profile obtained from MCM-41 and MCM-48 as well as TPA-MCM-41 and TPA-MCM-48 has been compared to see the effect of structural properties and geometry of carrier on release rate and results are shown in table 1. 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 1. 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 further selected for in-vivo study.

In vivo release study of CPT/MCM-48 and CPT/TPA-MCM-48

Experiment

Preparation of working curve for determination of Camptothecin

(a) Calibration in Chloroform-Methanol mixture

A stock solution of Camptothecin was prepared by dissolving 10 mg of standard CPT in chloroform methanol mixture (4:1), and making up the volume to 10 ml with Chloroform methanol mixture to give a final concentration of 1 mg/ml. From this stock solution, working standard solutions of 100 μg/ml was prepared by transferring 1 ml of standard stock solution to 10 ml volumetric flasks and adjusting the volume. From this working standard solution, second working standard solutions of 10 μg/ml was prepared by transferring 1 ml of standard stock solution to 10 ml volumetric flasks and adjusting the volume.

From this working standard solution 200, 400, 600, 800 and 1000 ng/ml was prepared. These solutions were analyzed and a standard curve was obtained by plotting the concentration of CPT versus the intensity of fluorescence (Figure 1).

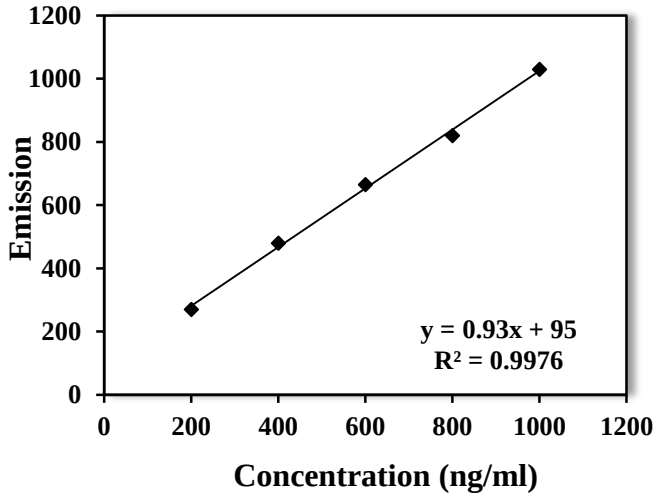

Figure 1. Calibration of Camptothecin in Chloroform-Methanol mixture

(b) Calibration in Blood plasma mixture

Stock solution

To prepare primary stock solution 10 mg of CPT was transferred into 10 ml of volumetric flask. Add 10 ml Chloroform and Methanol (8:2) and sonicated for about 5 min and volume made upto 10ml with methanol (1000µg/ml).

Primary and secondary stock solutions

The primary stock solution was prepared from stock solution, 1ml of stock solution was transferred into 10mL volumetric flask and volume made up to 10 ml with Acetonitrile.

The secondary stock solution was prepared from primary stock solution, 1ml of stock solution was transferred into 10mL volumetric flask and volume made up to 10ml with Acetonitrile.

Plasma sample preparation

0.1 ml of from secondary stock solution of each concentration was added in 2 ml polypropylene centrifuge tube. Add 0.90 ml of drug-free human plasma. Mixed the plasma and drug solution thoroughly for 5 minutes on vortex shaker.

Sample extraction procedure

Protein precipitation technique was used for drug extraction from plasma sample. From each centrifuge tube 0.5 ml of drug contained plasma was taken in 5 ml centrifuge tube and added 2 ml of acetonitrile contained 10 mM acetic acid was added to precipitate plasma proteins. Mix thoroughly for 5 minutes on vortex shaker. Then Centrifuge tubes were centrifuged at 10000 RPM for 10 min in high speed homogenizer (Sigma Hi Speed Homogenizer, Japan). Supernatant from each tube was separated using micropipettes and solution was analyzed by spectroflurometer. The obtained curve was shown in Figure 2.

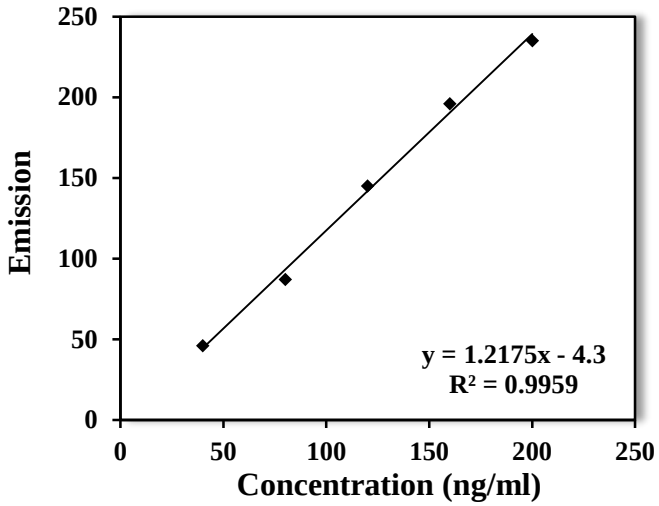

Figure 2. Calibration curve of Camptothecin in Blood plasma

Animal study

Animals

The in vivo studies were performed on male Wistar rats (200-300 g) and procured from Pretox Research Centre, Surat, India. The protocol for the study was duly approved by Institutional Animal Ethics Committee, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Vadodara, vide protocol approval no: MSU/IAEC/2017-18/1729. All experimental procedures were carried out as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines released by Ministry of Environment, Forests and Climate Change, Govt of India. The animals were housed in polypropylene cages under laboratory conditions of controlled environment of temperature 25 ± 2 °C, 60 ± 5% RH and 12 h dark/light cycle. Three animals per cage were fed ad libitum with animal feed allowing free access to drinking water. After acclimatization, the animals were randomly allocated to groups having six animals in each group for pharmacokinetic study**.**

In vivo release and Pharmacokinetic study

In vivo release profile and pharmacokinetic profile of CPT/MCM-48 and CPT/TPA-MCM-48 were compared.

Animal Dose calculation

Animal Equivalent dose was calculated using the following formula:

$$AED\ (mg/kg) = HED\ (mg/kg) * \frac{Human\ Km}{Animal\ Km}$$

Where,

AED = animal equivalent dose

HED = human equivalent dose

For CPT = 5 mg/kg of rat

The average weight of rats was considered 250 g. So, the orally administered dose of CPT was found to be 1.25 mg [1].

Animal grouping

The animals were randomly allocated to each group as shown in Table 2. Each group contained three animals.

Table 2. Groups of animals (Rats) for pharmacokinetic study

Groups	Samples administered
Group 1	CPT/MCM-48 (oral)
Group 2	CPT/TPA-MCM-48 (oral)

Procedure

For pharmacokinetics, drug and formulations were administered to rats as grouped in Table 2 to obtain plasma concentration – time profile. The animals randomly divided in the 2 groups were abstained from food overnight before beginning of experiment. After oral administration using oral gavage, blood samples were withdrawn from the retro-orbital plexus using heparinized capillary at 1, 1.5, 2, 4, 8 and 12 h. The blood samples

collected in pre-heparinized tubes were centrifuged at 3600 rpm, 4 ºC for 10 min (Remi Centrifuge, India) to separate plasma. The plasma samples were analyzed for CPT content using florescence spectroscopy.

Data Analysis

The pharmacokinetic parameters were calculated using Kinetica software (Thermo Scientific™, Thermofisher.com). The maximum concentration (Cmax) and time to reach maximum concentration (Tmax) were obtained from the plasma concentration-time profile. The area under the curve (AUC) was calculated by the trapezoidal rule. The relative bioavailability was calculated using following equation (1) ….

$$\text{Relative Bioavailability} = \frac{[\text{AUC}]_a/\text{dose}_a}{[\text{AUC}]_b/\text{dose}_b} \text{ X } 100 \text{ \% } \text{…. Equation …(1)}$$

Results and Discussion

Pharmacokinetic study

The plasma drug concentration vs. time profile for CPT/MCM-48 and CPT/TPA-MCM-48 formulation is shown in Figure 3 and corresponding pharmacokinetic parameters are shown in Table 3. 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 of the prepared CPT/MCM-48 and CPT/TPA-MCM-48 showed a significant difference from the pharmacokinetic profiles of pure CPT. The AUC of CPT/MCM-48 and CPT/TPA-MCM-48 in rats were 9802.13 ± 30.2 and 1358.52±30.2 ng/Lh, which was significantly improved as compared to that of pure CPT (187.80±58 ng/Lh). The improved AUC of CPT/MCM-48 and CPT/TPA-MCM-48 are due to more uptake of CPT.

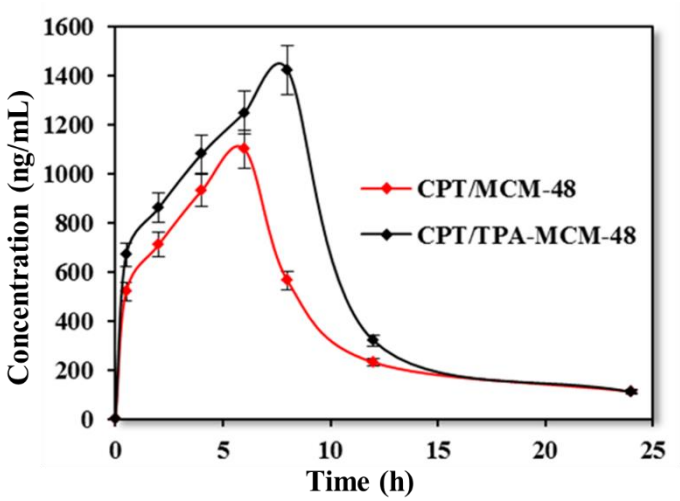

Figure 3. Concentration of CPT in plasma after oral administration of CPT/MCM-48 and CPT/TPA-MCM-48

Table 3. 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

Reference

[1] M. Manikandan, K. Kannan, As. J Pharm. Clin. Res. 9, 3, 71 (2016)