CHAPTER 5

In vitro release study of Amino acids (L-Arginine and Cysteine) from MCM-48 and TPA-MCM-48

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Cysteine and *N*-acetyl cysteine encapsulated mesoporous silica: synthesis, characterization and influence of parameters on in-vitro controlled release

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Abstract Pro-drug, cysteine loaded mesoporous silica materials with hexagonal (MCM-41) and cubic (MCM-48) geometry were synthesized by incipient wetness and soaking technique. The structure and properties of these synthesized materials were investigated by various physico-chemical techniques such as FT-IR, Nitrogen adsorption-desorption, XRD and TEM. An in-vitro release study of cysteine from these synthesized materials in SBF was carried out under stirring as well as static conditions. Effect of synthesis method as well as effect of geometry of carrier on release profile of drugs was also examined. Based on the obtained results for pro-drug, controlled release of real drug, N-acetyl cysteine was also carried out under optimized conditions. Release results shows that N-acetyl cysteine release was found to be more controlled as compare to that of cysteine from both mesoporous carriers. A study on release mechanism and release kinetics was also carried out using Higuchi model and first order release kinetic model.

Keywords MCM-41 · MCM-48 · Cysteine · *N*-acetyl cysteine · Controlled release · Kinetics

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1 Introduction

The construction of controlled-release systems for targeted drug delivery is of crucial importance for the development of both fundamental science and clinical medicine. In search of optimum drug delivery systems, variety of materials such as polymers [1], polymer based composites, bioactive glasses or ceramics [2, 3] has been frequently investigated. However, traditional disadvantage associated with mentioned systems i.e. disparate distribution of drug through these matrices, are clearly satisfied by mesoporous silica materials (M41S) as they possess ordered mesopores, high surface area, and well defined structure [4–7]. Studies also revealed that M41S have found potential applications for encapsulating bioactive molecules [8–11] and in domain of M41S family, extensive work has been carried out on MCM-41 as drug delivery carrier [12]. Among different bioactive molecules, release studies of ibuprofen from unfunctionalized and/or functionalized MCM-41 have been explored widely [13–19]. In spite of these, release of other molecules such as bisphosphonate [20], cytochrome C [21], cisplatin [22], Sulfadiazine [23], nifedipine [24], amino acids [25] from MCM-41 has also been reported.

The given reports suggested that factors such as the solubility of the drug in the solvent, the diffusivity of the drug in the solvent and the structure characteristics of the pore materials, pore diameter can seriously affect the release behaviors of the drug molecule. It was also shown that one-dimensional (1D) or three-dimensional (3D) cage-like pore structure with of mesoporous silica is of great benefit to control drug release [15]. In spite of these facts, less attention has been paid to other ordered mesoporous silica materials having different geometry e.g. cubic MCM-48 which contains three-dimensional channels. Qiu et al. have studied the release profile of

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In vitro release of L-arginine and cysteine from MCM-48: a study on effect of size of active biomolecules on release rate

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Abstract
The present paper consists of encapsulation of L-arginine as well as cysteine into MCM-48 with different amount and its characterization using various physicochemical techniques such as TEM, BET surface area, FT-IR, TGA, Powder XRD and ^{29}Si MAS NMR. The in-vitro release study of L-arginine as well as cysteine was carried out in simulated body fluid under static as well as stirring condition. A study on release kinetic as well as mechanism was also carried out using first ordered release kinetic model. The release profile of L-arginine and cysteine was co-related with the size and structure of biomolecules.

Keywords MCM-48 · L-arginine · Cysteine · In vitro-release · Effect of size and structure · Release kinetics and mechanism

1 Introduction

L-arginine and cysteine are essential amino acids. L-arginine involved in many biological process such as wound healing, tissue repaired, nitric oxide production etc., [1–12] and L-arginine intravenously administered to the patients with coronary artery disease to increase vascular NO bioavailability which show the vasodilatory effect. But the oral administrations of arginine do not show this effect. It is possible that the local availability of L-arginine as substrate for NO synthase may be reduce by the activity of arginase. Arginase utilizes L-arginine for the production of urea and ornithine and thus competes with NO synthase for substrate availability [3]. After administration of L-arginine, 40% is degraded in the intestine by arginase [4]. Cysteine is sulfur containing amino acid, acts as an antioxidant, and also involved in the production of glutathione. Reduction of this glutathione contributes to chronic inflammatory conditions, which are associated with cancer, neurogenerative, cardiovascular and infertility diseases resulting in high demand of cysteine [13, 14]. Cysteine pro-drugs are used to treat Schizophrenia and reduce drug cravings [15]. The administration of cysteine derivative such as *N*-acetylcysteine has major drawback of high dosage which can provoke persistent damage and strong allergic reactions [16–20].

As mentioned earlier, 40% L-arginine is degraded after oral administration and also required minimum three doses per day. For cysteine, also high dosages are required which may cause strong allergic reaction. The mention problems can be overcome if these biomolecules can be delivering in slow doses with controlled manner. This can only possible by using proper delivery carrier.

As M41S family have properties like higher surface area, ordered porosity, higher adsorption capacity, biocompatibility and non-cytotoxicity, they have been effectively explored as drug delivery carrier [21–24]. Among them a number of reports are available on MCM-41 and SBA-15 with functionalized or unfunctionalized form [25–50].

At the same time, very few reports are available on MCM-48, same member of the M41S family as drug delivery carrier [34, 50–53]. Recently, Cheng zhong Yu and his group have reported the release profile of Sulfasalazine from MCM-48 [54]. Zink et al. have reported the comparative study for release of hydrophilic dye (Rose bangal) and hydrophobic molecule [Camptothecin (CPT) and Rhodamine 6G (R6G)] from MCM-48 [55] Daniela Berger et al. have reported comparative study for release of aminoglycoside from MCM-48 [49].

As stated earlier, although L-arginine and cysteine are very important active biomolecules, only two reports are available: (1) adsorption of L-arginine on SBA-15 at different

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EXPERIMENTAL

In vitro controlled release of L-Arginine

In vitro release profile of L-Arginine was obtained by same method as stated in chapter 2. Release study was carried out under different condition (stirring, static as well as different pH).

Results and discussion

(a) Effect of stirring on release rate of L-Arginine

In vitro release profile of L-arg/MCM-48 and L-arg/TPA-MCM-48 in SBF under static and stirring condition at 37 ^{o}C was carried out and results are shown in Figure 1. For L-arg/MCM-48, initially 52% of L-Arginine release and reached to 69% in 10 h. After that slow and delayed release is observed and reached up to 86% in 30 h. however, under static condition slower release is observed.

For L-arg/TPA-MCM-48, initially, 28% of L-Arginine is released under both the condition but after that slower release is observed under static condition. It reached up to 51% and 38% in 10 h and 86% and 46% in 32 h under stirring and static condition respectively. The slower release of L-Arginine is may be due to the slow diffusion of L-Arginine molecules under static condition.

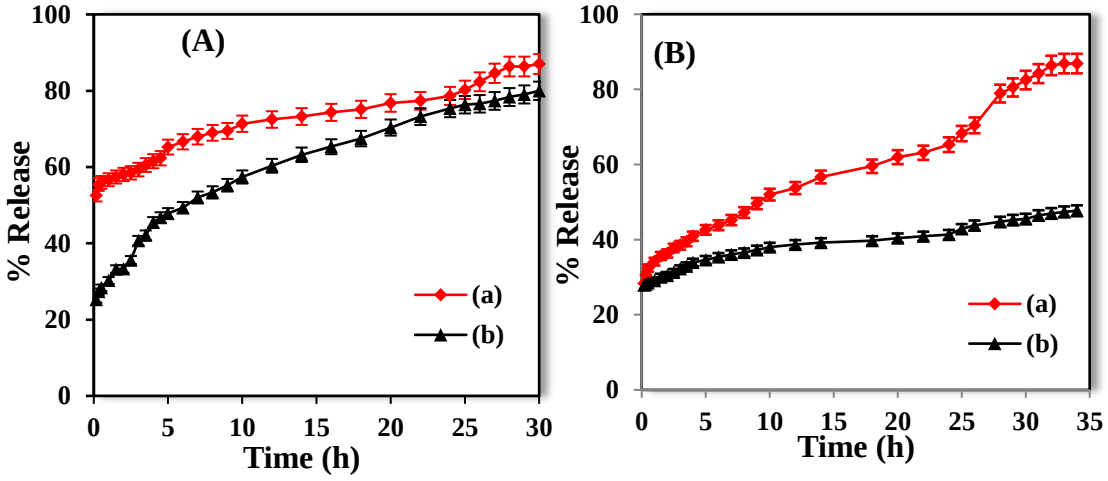

Figure 1. In vitro Release profile of (A) L-arg$_1$/MCM-48 and (B) L-arg$_1$/TPA-MCM-48 under (a) stirring and (b) static condition

(b) Effect of pH on release rate of L-Arginine

To see the effect of pH on release rate, release study was carried out pH 7.4 and pH 1.2. Figure 2 shows release profile of L-arg/MCM-48 and L-arg/TPA-MCM-48 at different pH. In acidic pH slow release was observed for both systems. L-Arginine acquires two positive charges and remains as Arg^{2+} which may have strong interaction with carrier which will slow down the diffusion of L-Arginine molecules. While at pH 7.4, L-Arginine remains as Arg^{+} which has comparatively weak interaction which allows easy diffusion.

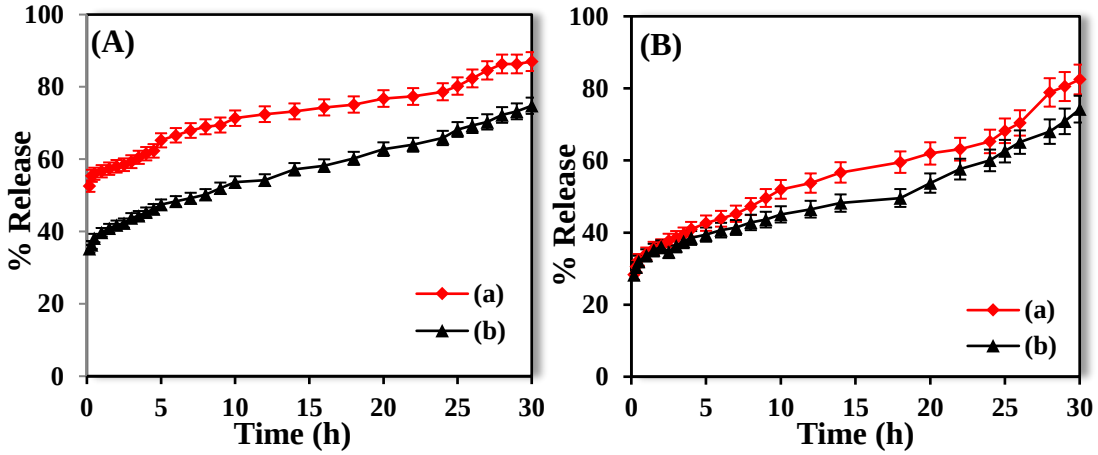

Figure 2. In vitro release profile of (A) L-arg_1/MCM-48 and (B) L-arg_1/TPA-MCM-48 in (a) SBF (pH 7.4) and (b) SGF (pH 1.2)

(c) Effect of TPA on release rate of L-Arginine

To see the effect of TPA on release rate of L-Arginine, release profile of L-arg/MCM-48 and L-arg/TPA-MCM-48 have been compared and results are shown in Figure 3. It is clear from the Figure 3 that slower release profile is obtained for TPA-MCM-48 compared to pure MCM-48. In case of L-arg/MCM-48, initially 52% of L-Arginine was released and reached up to 69% in 10 h. while in case of L-arg/TPA-MCM-48, initially 28% L-Arginine released and reached up to 51% in 10 h. Thus, more controlled and ordered release rate is observed with TPA-MCM-48 systems compared to pure MCM-48. The slower release of L-Arginine may be due to the strong interaction between L-Arginine and terminal oxygen of TPA, which was already confirmed from FT-IR spectra. Further, the pore volume of L-arg/MCM-48 is bigger than L-arg/TPA-MCM-48. So there may be easy diffusion of L-Arginine molecules from MCM-48 compared to TPA-MCM-48. Here, TPA act as functionalizing agent and can hold the L-Arginine for longer period of time.

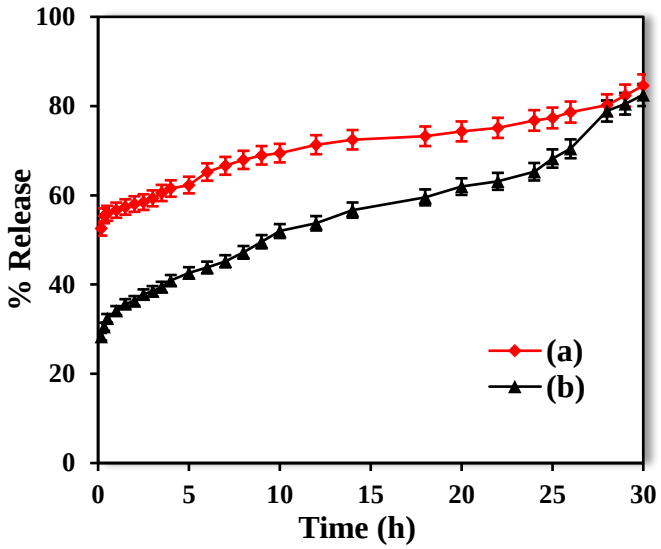

Figure 3. Comparison of in vitro release profile of (a) L-arg/MCM-48 and (b) L-arg/TPA-MCM-48

Further, to see that TPA is actually acting as functionalizing agent or not, FTIR analysis of L-arg/TPA-MCM-48 was carried out after release study and spectrum is shown in Figure 4. It shows that bands correspond to N-H stretching vibration and CH_3 in plan bending vibration are disappeared. This may be due to the removal of L-Arginine from TPA-MCM-48 during the release study. However the bands correspond to NH_2 in plan bending vibration and C=O stretching vibration show slight shifting with lower intensity. This suggests that some amount of L-Arginine was remaining inside the TPA-MCM-41. That was also confirm from release study as it shows that 86% L-Arginine was release up to 32 h and then it became constant. Further, bands corresponding to TPA-MCM-48 are remaining as it is and confirm the intact structure of TPA-MCM-48.

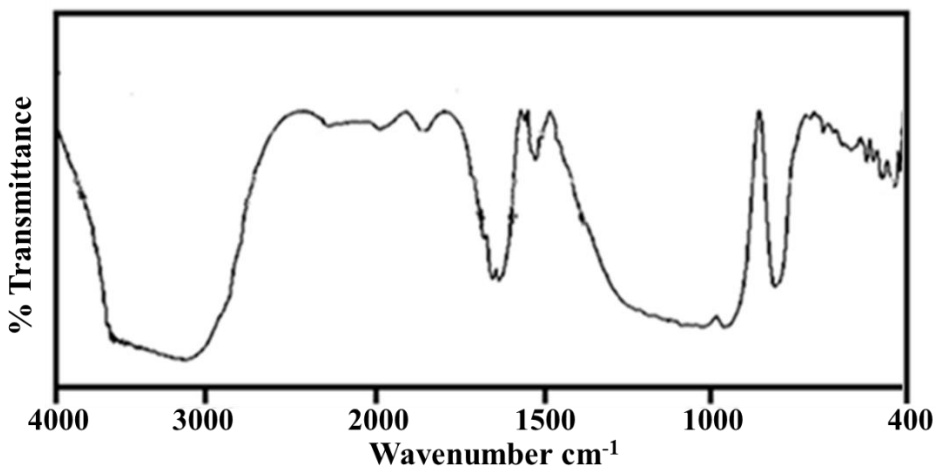

Figure. 4. FT-IR spectrum of L-arg/TPA-MCM-48 after release study

Kinetics and Mechanism

In order to understand the release kinetics of L-Arginine, the drug release data of all systems were fitted with first order release kinetics model and Higuchi model.

(i) First order release kinetic model

Figure 5 shows first order release kinetic model of L-arg/MCM-48 and L-arg/TPA-MCM-48 kinetic parameters are shown in Table 1. The release of L-Arginine follows the first order release kinetic model with higher linearity and co-relation coefficient for L-arg$_1$/TPA-MCM-48 (R^2 = 0.9903).

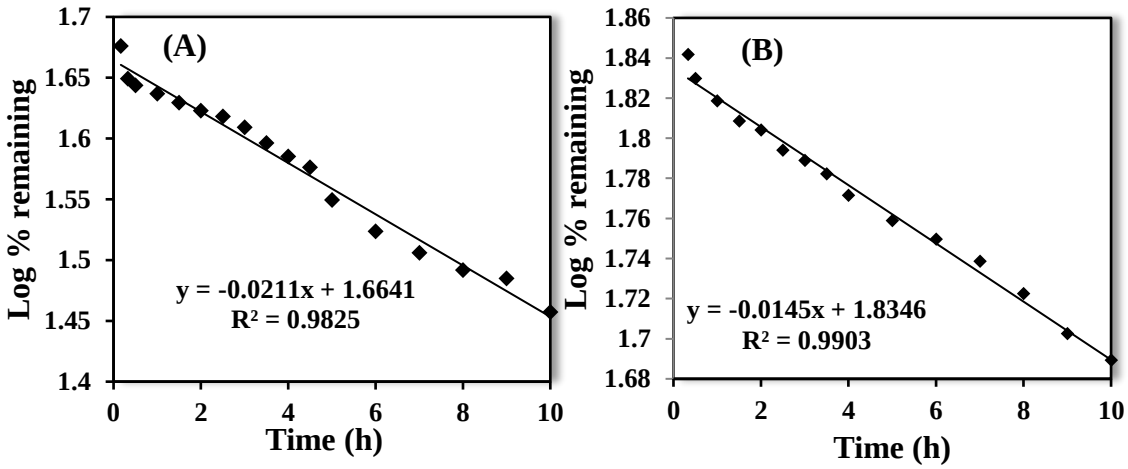

Figure 5. First order release kinetic model for (A) L-arg/MCM-48 and (B) L-arg/TPA-MCM-48

(ii) Higuchi Model

Figure 6 shows Higuchi model for L-arg/MCM-48 and L-arg/TPA-MCM-48 where % release of L-Arginine was plotted against square root of time based on Fickian diffusion mechanism. According to this model release mechanism involves simultaneous penetration of SBF into the pores of carriers, dissolution of drug molecules and diffusion of these molecules from the carriers. This release data up to 10 h was best fitted with Higuchi model with higher linearity and co-relation coefficient (R^2 = 0.9863) for L-arg/TPA-MCM-48and follows Fickian diffusion mechanism.

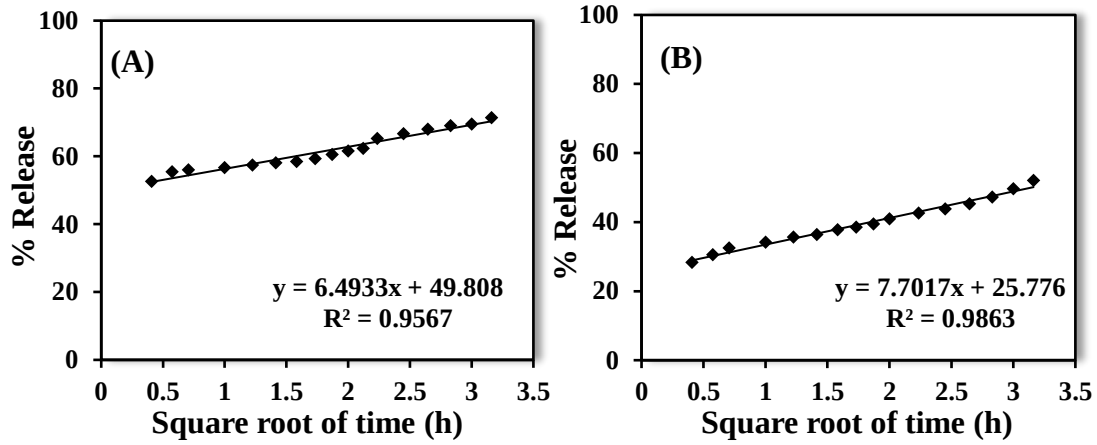

Figure 6. Higuchi model for (A) L-arg/MCM-48 and (B) L-arg/TPA-MCM-48

Hence release profile of L-Arginine follows first order release kinetic model as well as Higuchi model.

Table 1. 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 R_1 = 0.982	K_1 = 0.014 R_1 = 0.990
Higuchi model	K_2 = 6.49 R_2 = 0.956	K_2 = 7.70 R_2 = 0.986

In vitro controlled release of Cysteine

The release profile of Cysteine was obtained by soaking the Cysteine loaded materials in 150 ml of a simulated body fluid, SBF (1 mg of the Cysteine sample per ml of fluid), and measuring the drug concentration in the fluid by means of a Uv-vis spectrophotometer (PerkinElmer Lambda 35) at 570 nm. At predetermined time interval released fluid was taken (0.5 mL) and immediately equal amount of fresh SBF was added to maintain the constant volume. This release fluid was analyzed for L-Arginine content by treating it with 10 % ninhydrin solution at 570 nm using (systronic) UV–Visible spectrophotometer. All the experiments were repeated three times. Further release study was carried out in gastric fluid (GF) at body temperature and under stirring condition.

Results and Discussion

a) Effect of loading method

To evaluate the effect of loading method, release profile of Cysteine loaded materials using soaking method and incipient wetness impregnation method are compared and results are shown in Figure 7. Initially 34% and 51% of Cysteine is released in case of Cys/MCM-48 and Cys/MCM-48(I) respectively. Further slow and delayed release is observed for Cys/MCM-48. It is expected that in case of incipient wet impregnation method, 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, the burst and fast release of loosely held Cysteine on the outer surface of MCM-48 is observed for Cys/MCM-48(I). Also, controlled release in the case of Cys/MCM-48 indicates presence of relatively firmly bound Cysteine molecules inside the channels of MCM-48 during the soaking method. In vitro release study of Cysteine shows that more controlled release rate for Cys/MCM-48. Hence, Cysteine loaded into TPA-MCM-48 by soaking method only (Chap-1).

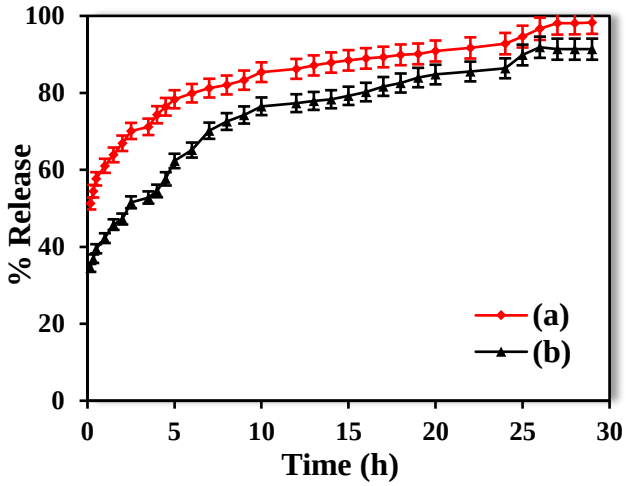

Figure 7. In vitro release profile of (a) Cys/MCM-48(I) and (b) Cys/MCM-48

(b) Effect of stirring on release profile of Cysteine

In order to evaluate the effect of stirring on release rate of Cysteine, in-vitro release of Cysteine was carried out under stirring condition as well as static condition and results are shown in Figure 8. Both the systems, Cys/MCM-48 (Figure 8A) and Cys/TPA-MCM-48 (Figure 8B) show slower release under static condition. In case of Cys/MCM-48, initially 28% of Cysteine is released under static condition while 34% is released under stirring condition. After that controlled release of Cysteine is observed and reached up to 70% and 91% in 30 h under static and stirring conditions respectively.

Similar observation is obtained for Cys/TPA-MCM-48. Initially, 25% and 28% of Cysteine is released under static and stirring condition respectively. After that, release of Cysteine, in controlled manner is observed in stirring condition and reached to maximum at 30 h. (Figure 8B). The observed slower release of Cysteine under static condition is may be due to the slower diffusion of Cysteine which eventually decreases the dissolution rate from materials to the release medium and thus required high time for complete release of Cysteine. More convenient result is obtained under stirring condition. So, further release profile of these materials as compared.

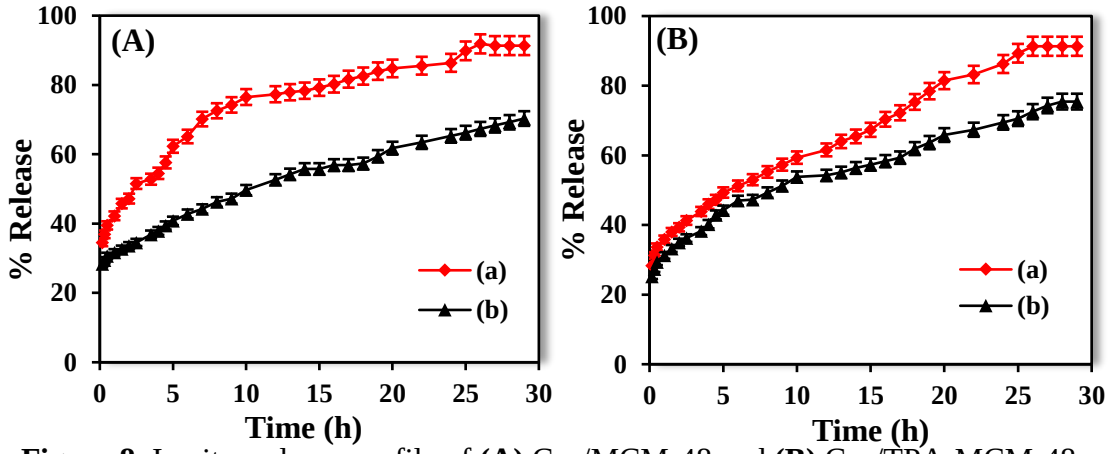

Figure 8. In vitro release profile of **(A)** Cys/MCM-48 and **(B)** Cys/TPA-MCM-48 under (a) stirring and static (b) condition

(c) Effect of TPA on release rate of Cysteine

To see the effect of TPA on release rate of Cysteine, release profile of Cys/MCM-48 and Cys/TPA-MCM-48 were compared and results are shown in Figure 9. Initially, 34% and 28% of Cysteine is released and reached up to 76% and 59% in 10 h from MCM-48 and TPA-MCM-48, respectively. It reached up to 91% in 30 h for MCM-48 and TPA-MCM-48, respectively. Here, slower release profile is obtained for Cys/TPA-MCM-48 as compared to Cys/MCM-48.

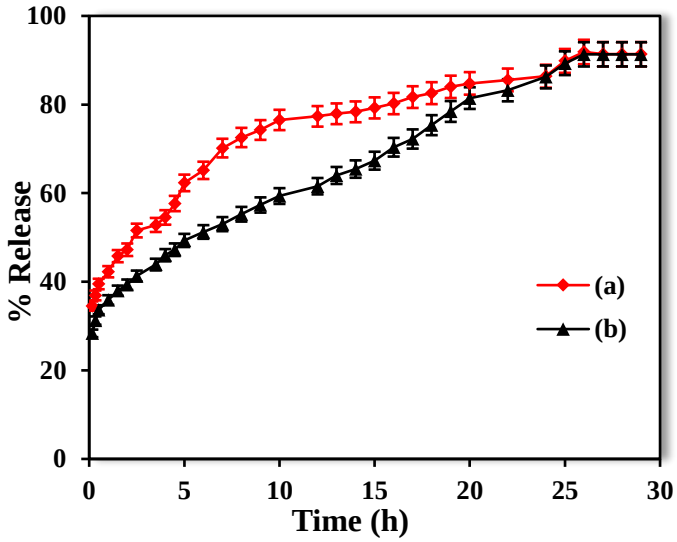

Figure 9. Comparison of release profile of (a) Cys/MCM-48 and (b) Cys/TPA-MCM-48

This is may be because of the more attractive interaction between the Cysteine 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 Cysteine from TPA-MCM-48.

FTIR of Cys/TPA-MCM-48, after release study was also carried out for finding that the TPA is truly act as functionalizing agent and spectrum is shown in Figure 10. Spectrum is similar to that of TPA-MCM-48 and show entire bands related to TPA-MCM-48. Further, absence of any splitting in band of P-O bond suggests the intact structure of TPA-MCM-48. Along with this, bands corresponding to Cysteine are disappear which is due to the removal of it during release study.

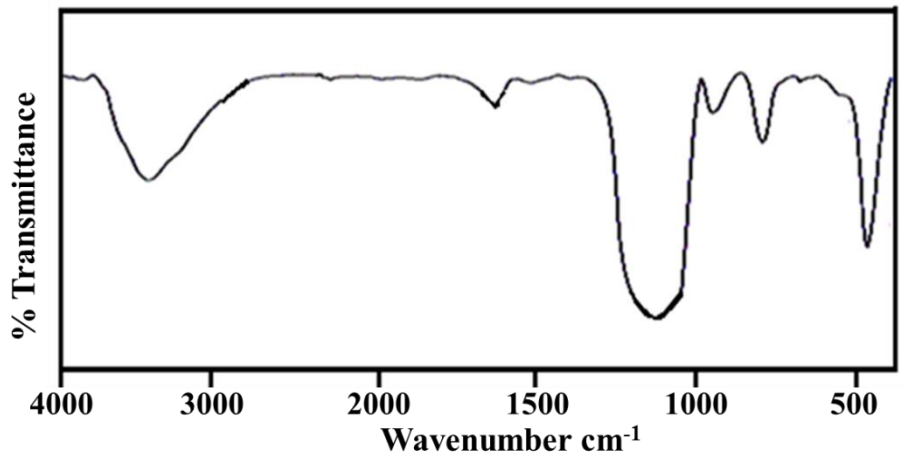

Figure 10. FTIR of Cys/TPA-MCM-48 after release study

Kinetics and mechanism

(a) First order release kinetic model

In order to analyze Cysteine release in a detail and obtain the possible release mechanism, release data up to 10 h is fitted with first order release kinetic model and kinetic parameter are shown in Table 2. Figure 11 shows the first order release kinetic model of Cys/MCM-48, Cys/MCM-48(I) and Cys/TPA-MCM-48 where Log of percentage remaining data is plotted against time. The release of Cysteine follows the first order kinetic with linearity and correlation coefficient (R^2) value for Cys/TPA-MCM-48 (0.9963) as compared to Cys/MCM-48 (0.9948) and Cys-MCM-41(I) (0.9565).

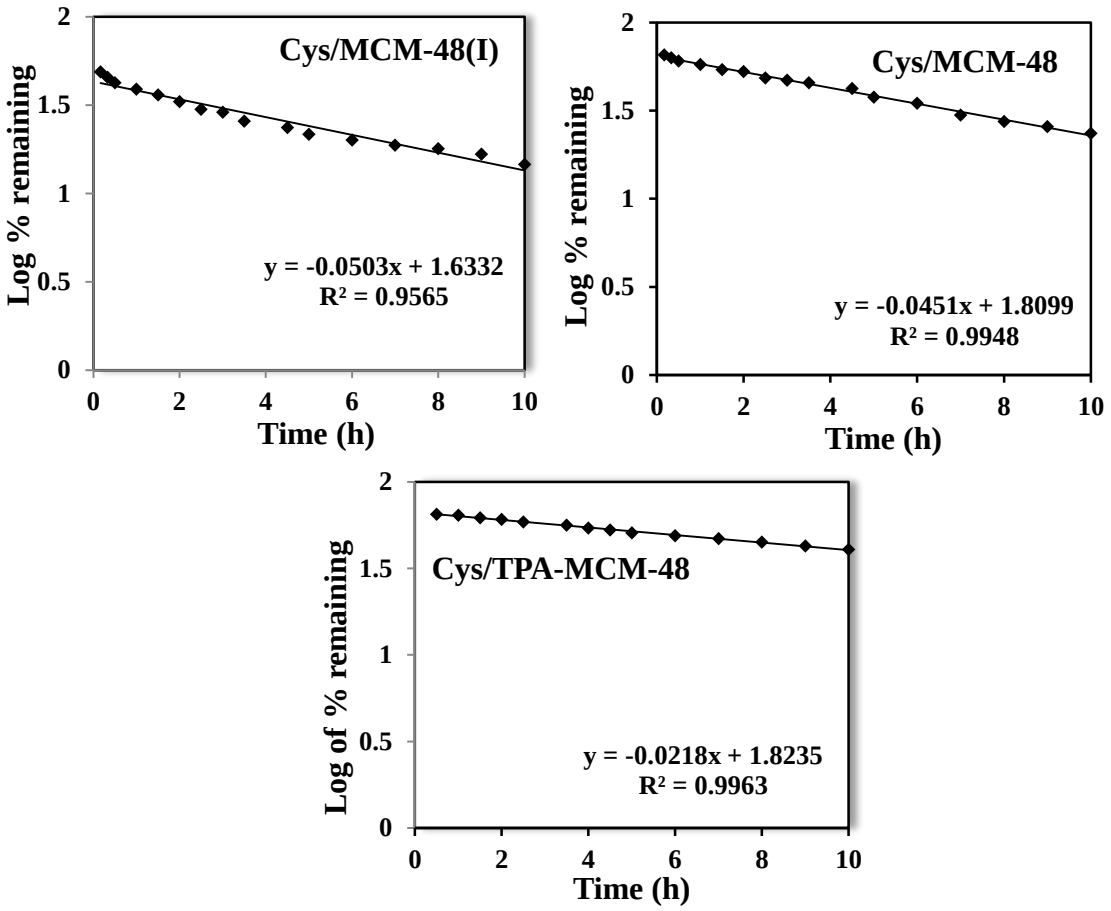

Figure 11. First order release kinetic model of Cys/MCM-48, Cys/MCM-48(I) and Cys/TPA-MCM-48

(b) Higuchi Model

The Higuchi model (Figure 12) describes the percentage release versus square root of time dependent process based on Fickian diffusion. According to this model release mechanism of Cysteine involves simultaneous penetration of SBF into the pores, dissolution of drug molecule and diffusion of these molecules from the pores. The in vitro Cysteine release data is best fitted with Higuchi model. The release mechanism of Cysteine is best explained by this model with high linearity and high correlation coefficient (R^2) value for Cys/TPA-MCM-48(0.9962) compared to Cys/MCM-48 (0.9915) and Cys/MCM-48(I) (0.977).

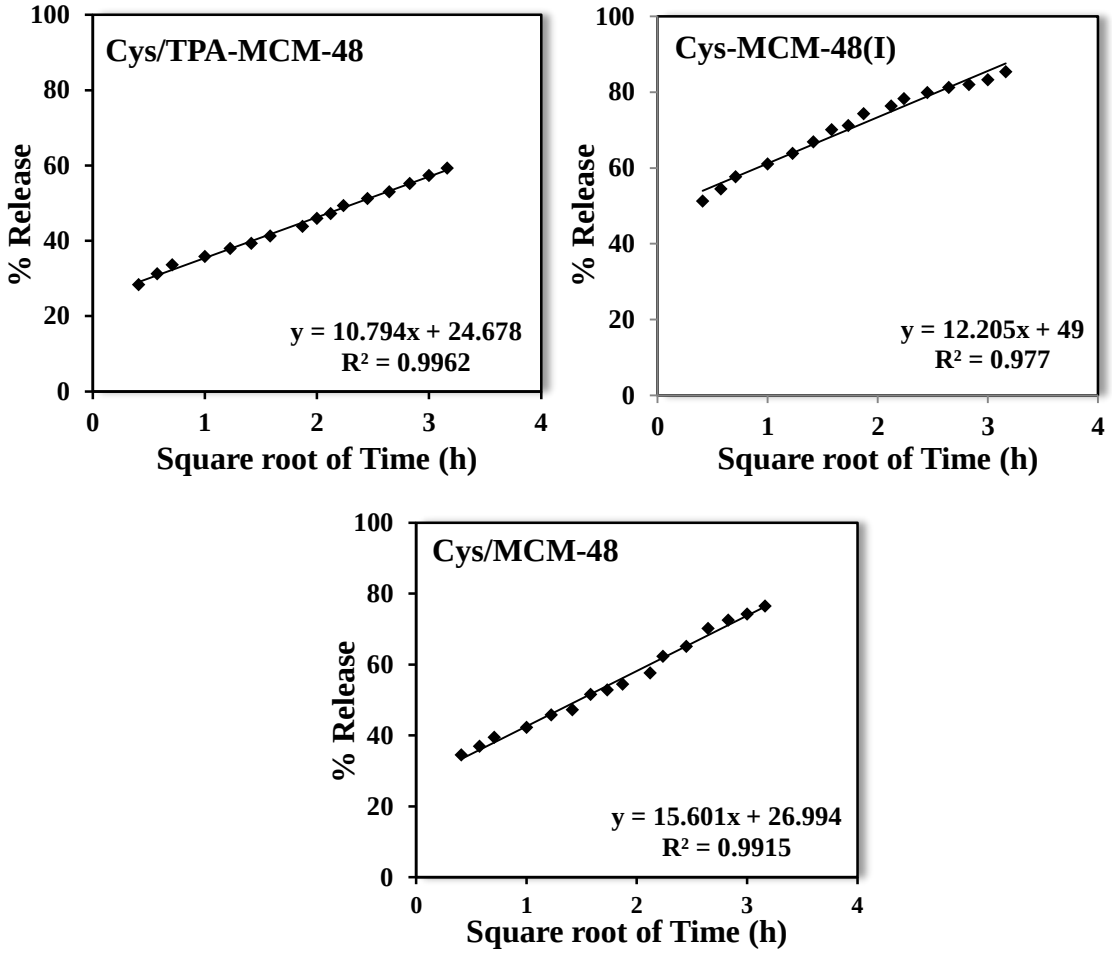

Figure 12. Higuchi model of Cys/MCM-48, Cys/MCM-48(I) and Cys/TPA-MCM-48

Here, more ordered release data is obtained for Cys/TPA-MCM-48 (Table 1). Thus, Cysteine release from these systems is concentration dependent process and follows first ordered release kinetics and the Fickian diffusion mechanism.

Table 2. 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 R_1 = 0.994	K_1 = 0.050 R_1 = 0.956	K_1 = 0.021 R_1 = 0.996
Higuchi model	K_2 = 15.60 R_2 = 0.991	K_2 = 12.20 R_2 = 0.977	K_2 = 10.79 R_2 = 0.996

Conclusions

- In vitro release study of L-Arginine as well as Cysteine shows that stirring has great influence on release rate. Under static condition slower release was observed because of slower diffusion of L-Arginine/Cysteine.
- Further, study shows that at lower pH slow release of L-Arginine as it shows more interaction with carrier at lower pH.
- To see the effect of loading method on release rate, release profile of Cysteine loaded by different method (wet impregnation and soaking) has been compared and result shows that more ordered and slow release was obtained for Cys/MCM-48 as compared to Cys/MCM-48(I).
- Further, study shows that TPA has great influence on release rate of L-Arginine as well Cysteine. TPA has terminal oxygen through which it binds with L-Arginine/Cysteine and holds it for longer period of time and shows slower release.
- FT-IR after release study, for both system (L-arg/TPA-MCM-48 and Cys/TPA-MCM-48) suggest that TPA is truly act as functionalizing agent.
- Kinetic and mechanistic study of L-Arginine/Cysteine shows that it follows first order kinetic and diffusion mechanism.