CHAPTER 2

In vitro release study of Amino acids (L-Arginine and Cysteine) from MCM-41 and TPA-MCM-41

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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

Introduction

L-Arginine is a semi essential amino acid. It is very important active biomolecule involved in many biological processes. Under certain condition, L-Arginine supplementation has been given for restoring normal function. For example L-Arginine is intravenously administrated to the patients with coronary artery disease to increases the bioavailability of nitric oxide (NO) which shows vasodilator effect. However, it was found that 40% L-Arginine is degraded in the intestine by arginase. Hence, its oral administration using proper carrier can minimize the mentioned problem.

Thus, even though, L-Arginine is important and is used as drug under certain conditions, very few reports are available. Qiang Gao et al. have reported the adsorption of L-Arginine on SBA-15 at different pH [1]. Lacasta et al.have reported synthesis of chiral ordered mesoporous silica in presence of L-Arginine and different amino acid to induce chirality in mesoporous silica and use it for chiral resolution [2]. Diaz et al., have reported immobilization of trypsin into MCM-41 which was active for the hydrolysis of N-a-benzoyl-DL-Arginine-4-nitroanibde (BAPNA) [3]. Casado et al. have reported synthesis of chiral ordered mesoporous silica in the presence of amino acid proline by combining tetraethyl orthosilicate and quaternized aminosilane silica sources for resolution of proline racemate [4]. However, no report is available on controlled release of L-Arginine.

Cysteine is a sulfur-based amino acid, itself can act as an antioxidant in the body. Cysteine pro-drugs are used to treat Schizophrenia and reduce drug cravings. However, the major drawback of Cysteine therapy is its high dosages that can provoke persistent damage and strong allergic reactions. Although, Cysteine is very important active biomolecules, only one report is available by Victor S-Y Lin and his group [5]. They have reported release study of Cysteine through mesoporous silica nanoparticle.

In this chapter, we have described the in vitro controlled release of L-Arginine and Cysteine from MCM-41 and TPA-MCM-41. Release study of selected amino acids has been carried out at different pH (7.4 and 1.2), under stirring and static condition. Further, comparisons of release profile of materials synthesized by wet impregnation method and soaking method has been included. Detailed kinetic and mechanistic study of amino acid release has also been investigated using various models such as First order release kinetic model and Higuchi Model.

In vitro controlled release of L-Arginine

EXPERIMENTAL

Materials

Sodium chloride (NaCl), Sodium bicarbonate ($NaHCO_3$), Potassium chloride (KCl), Potassium hydrogen phosphate ($K_2HPO_4.3H_2O$), Magnesium chloride ($MgCl_2.\ 6H_2O$), HCl, Calcium chloride ($CaCl_2$), Sodium sulfate (Na_2SO_4) and Tris buffer $NH_2C(CH_2OH)_3$ were used as received from Merck.

Preparation of Simulated body fluid (SBF) and Simulated gastric fluid (SGF)

NaCl (7.996 g), $NaHCO_3$ (0.350 g), KCl (0.224 g), $K_2HPO_4.3H_2O$ (0.22 g), $MgCl_2.6H_2O$ (0.305 g), 1 $molL^{-1}$ HCl (40 mL), $CaCl_2$ (0.278 g), Na_2SO_4 (0.071 g) and $NH_2C(CH_2OH)_3$ (6.057 g) were dissolved in small amount of water and then dilute to 1 L with distilled water. The obtained solution was designated as SBF.

6.217 g of Conc. HCl was taken in a 1000 mL volumetric flask and then brought up to the mark with the distilled water. The obtained solution was designated as simulated gastric fluid.

In vitro controlled release of L-Arginine

(a) Preparation of Calibration curve for L-Arginine

Stock solution of L-Arginine was prepared by dissolving 1 g in 100 mL SBF (10 mg/mL). Series of 10 mL Nessler's tubes containing 0.15, 0.2, 0.25, 0.3, 0.35 and 0.4 mL of this stock solution was diluted up to the 4 mL using SBF. 1 mL of acetate buffer (pH 5.5) and 1 mL ninhydrin reagent (10 % ninhydrin solution in ethanol) was added to these solutions. All the tubes were put in boiling water bath up to 10-15 min for completion of reaction which was indicated by the formation of purple colour. The solutions were cooled under tap water and then 1 mL of 50 % ethanol was added to each of the solutions and absorption was taken at 570 nm wavelength using Systronics UV-Visible spectrophotometer**.** Calibration of L-Arginine in SGF (pH 1.2) was also carried out using the same method and the obtained calibration curves are shown in Figure 1.

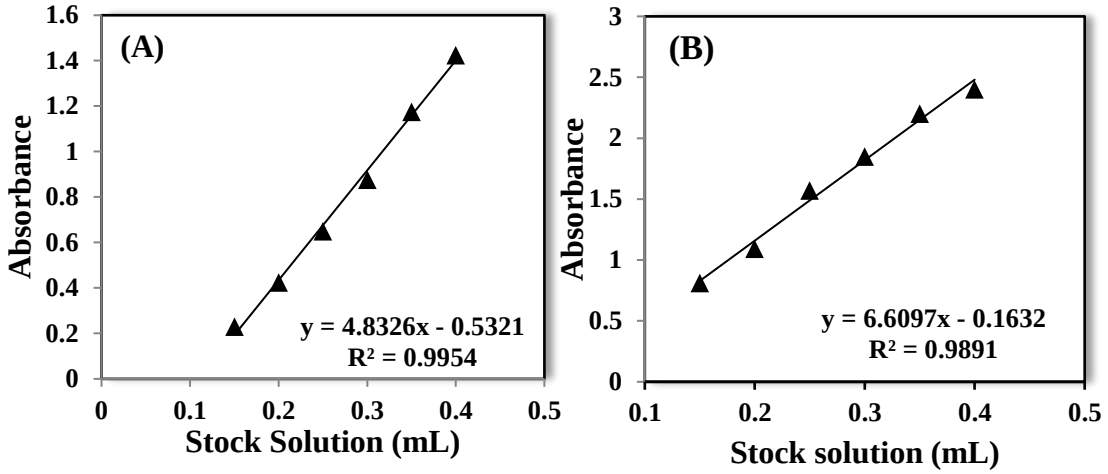

Figure 1. Calibration curve of L-Arginine in (A) SBF (pH 7.4) and (B) SGF (pH 1.2)

(b) In vitro controlled release of L-Arginine

In vitro release profile of L-Arginine was obtained by soaking 0.5 g of L-Arginine loaded materials in 62.5 mL of SBF (0.8 mg of L-Arginine in 1 mL SBF) at 37 ^{o}C temperature and at 200 rpm. At predetermined time interval, 0.5 mL of released fluid was taken and immediately equal amount of fresh SBF was added to maintain the constant volume. The first fraction (released fluid) was taken at 10 min (0.166 h). This release fluid was analyzed for L-Arginine content by treating it with 10 % ninhydrin solution at 570 nm using UV–Visible spectrophotometer (Perkin Elmer). Same study was also carried under static condition as well as at different pH (pH 7.4 and pH 1.2). All the experiments were repeated three times. For rest of the study also, first fraction (released fluid) was taken at 10 min (0.166 h).

Results and discussion

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

In order to see the effect of stirring on release rate of L-Arginine, in vitro release study was carried out under two different conditions: (1) stirring as well as (2) static and results are shown in Figure 2. Under stirring condition, in case of L-arg/MCM-41, initially 35% L-Arginine was released and reached up to 91% in 27 h while in case of L-arg/TPA-MCM-41 initially 25% of L-Arginine was released and reached up to 80% in 27 h. However, slower release was observed for both systems due to slower diffusion of L-Arginine under static conditions (Figure 2).

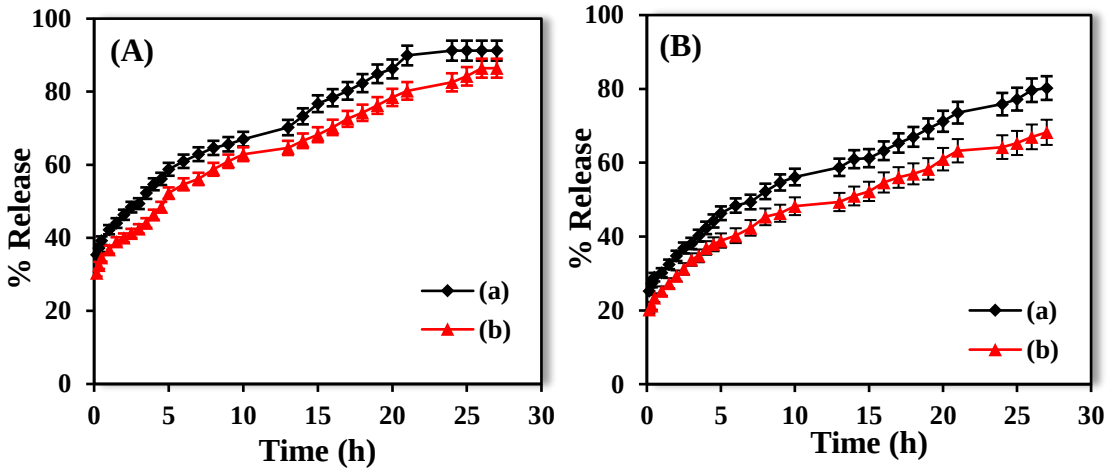

Figure 2. In vitro release profile of (A) L-arg/MCM-41 and (B) L-arg/TPA-MCM-41 under (a) stirring and (b) static condition

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

In order to see the effect of pH on release of L-Arginine, a comparative study was carried out in SBF and SGF and results are shown in Figure 3. In acidic pH slow release was observed, as L-Arginine acquires two positive charges and remain as Arg^{2+} [6]. These may have strong interaction with terminal oxygen of TPA moiety which will slow down the diffusion of L-Arginine molecules. On the other hand, at pH 7.4, L-Arginine remains as Arg^{+} which has comparatively weak interaction that allows the easy diffusion.

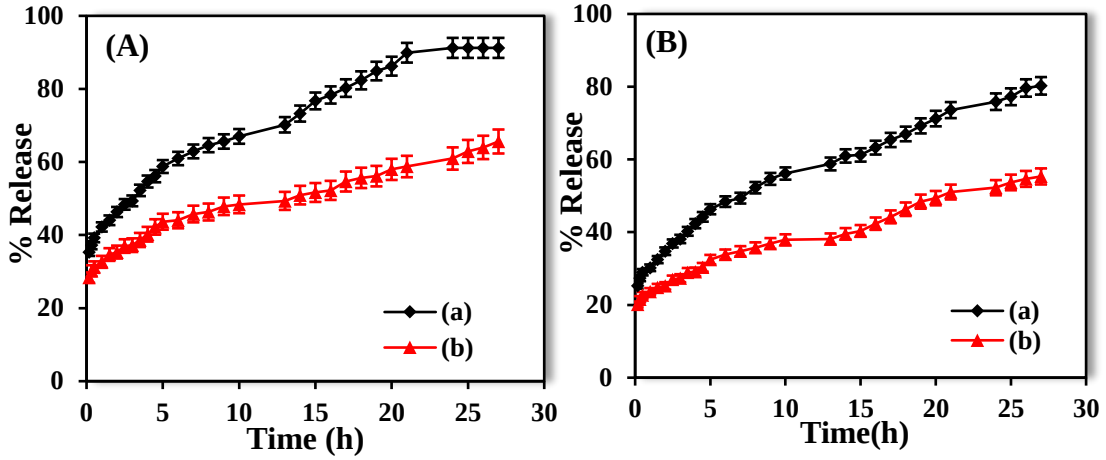

Figure 3. In vitro release profile of (A) L-arg/MCM-41 and (B) L-arg/TPA-MCM-41 in (a) SBF (pH 7.4) and (b) SGF (pH 1.2)

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

To see the effect of TPA on release rate, release profile of L-arg/MCM41 and L-arg/TPA-MCM-41 has been compared and shown in Figure 4. It is well known that the pore size [7–9] as well as the host guest interaction [10] has a pronounced influence on the kinetics of drug release. Drug delivery rate slows down with the decrease of the pore size. It also decreases with increase in the host–guest interaction i.e. stronger the interaction, slower is the drug delivery rate.

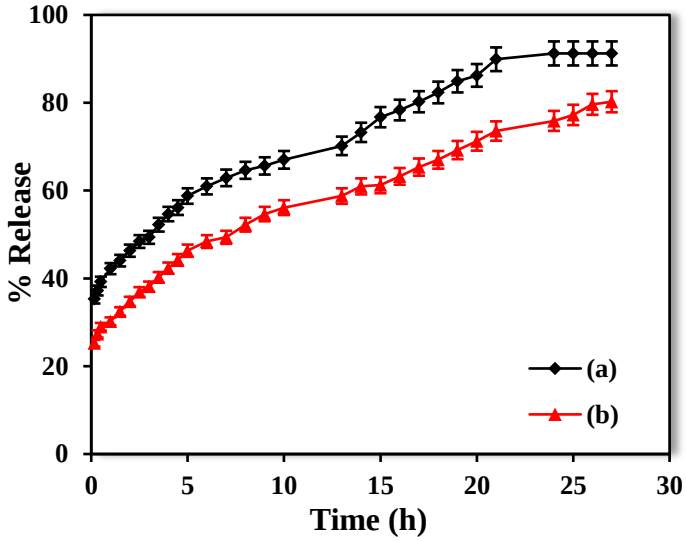

Figure 4. Comparison of in vitro release profile of (a) L-arg/MCM-41 and (b) L-arg/TPA-MCM-41 under stirring condition

In the present case, the slow release of L-Arginine may be due to the following reasons. (1) The strong interaction between L-Arginine molecules with oxygen of TPA, which was already confirmed by FTIR spectra (Chapter 1b, Figure 4). (2) Further, the

pore volume of L-arg/TPA-MCM-41 is smaller than the L-arg/MCM-41 (BET surface area analysis, Chapter 1A, Table 2 and Chapter 1B, Table 4). So there may be restricted diffusion of L-Arginine that occurred from smaller pore volume. The observed results are in good agreement with the reported literature [7–9].

FTIR analysis of L-arg/TPA-MCM-41 was carried out after release study and spectrum is shown in Figure 5. It is seen from the spectra that the bands correspond to N-H stretching vibration and CH_3 in plane bending vibration have disappeared. This may be due to the removal of L-Arginine from TPA-MCM-41 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 confirmed from release study as it shows that 80% L-Arginine was released up to 27 h and then it became constant.

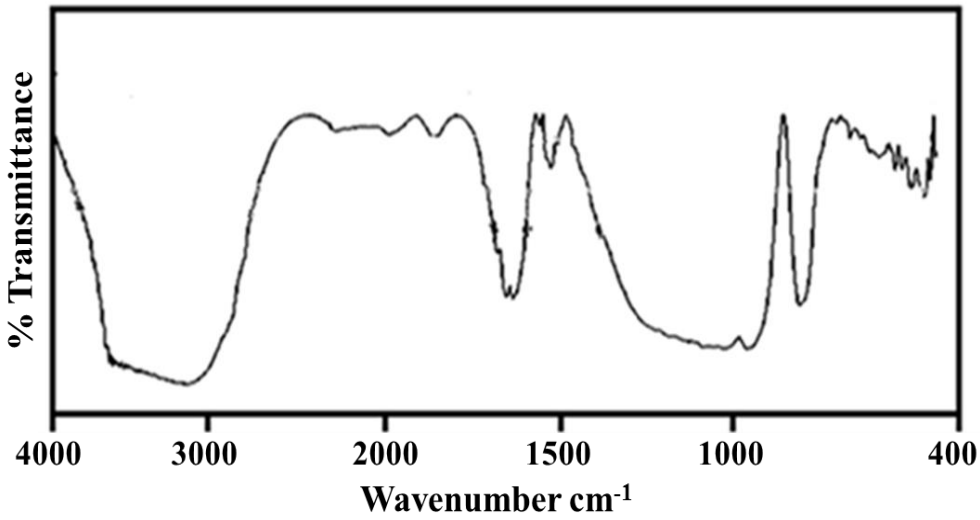

Figure 5. FTIR spectrum of L-arg/TPA-MCM-41 after release study

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 [11-13].

The first order release model describes the release of drug which is concentration dependent and applied for water soluble drugs (Eq. 1).

$$\text{Log } C_0 - \text{Log } C_t = kt/2.303 \quad (1)$$

where C_0 is the initial concentration of drug and Ct is the concentration of drug at time t. k is the first order release kinetic constant. The plot of log of % remaining versus time will be linear with the negative slope for this model.

The Higuchi mechanism describes the percentage of release versus square root of time dependent process based on Fickian diffusion (Eq. 2). This model was used to describe the drug release from granular spherical partials and follow the dissolution mechanism.

$$Q = KHt^{1/2} \quad (2)$$

where, Q is the amount of drug release, t is the time and KH is the Higuchi constant. This model shows the mechanism of drug release which involves the simultaneous penetration of SBF into the pore, dissolution of drug molecule into the SBF and diffusion of drug molecule through the pore.

(i) First order release kinetic model

Figure 6 shows the first order release kinetic model of L-Arginine release from MCM-41 and TPA-MCM-41 up to 10 h and the kinetic parameters obtained are shown in Table 1. The log percentage remaining was plotted against the time in hour. The release of L-Arginine follows the first order kinetic with higher linearity and higher correlation coefficient (R^2) value for L-arg/TPA-MCM-41(0.987) compared to L-arg/MCM-41(0.975). The release data for L-arg/TPA-MCM-41 sample was best fitted with first ordered release kinetic model (Figure 6).

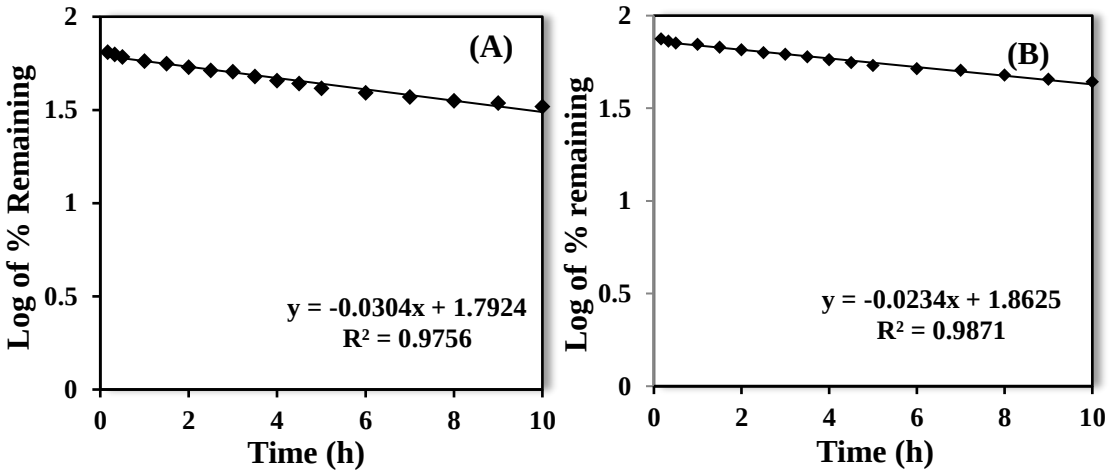

Figure 6. First order release kinetic model for (A) L-arg/MCM-41 and (B) L-arg/TPA-MCM-41

(ii) Higuchi Model

Figure 7 presents the Higuchi model of in vitro release of L-Arginine from MCM-41 and TPA-MCM-41.The release data for both the system was fitted with Higuchi model for finding the release mechanism. The percentages release data were plotted against the square root of time in h where linear curve is obtained. The release of L-Arginine follows the Higuchi model with higher linearity and higher correlation coefficient (R^2) value for L-arg/TPA-MCM-41 (0.9916) compared to L-arg/MCM-41(0.9902). The above, in vitro release and kinetic study shows that L-arg/TPA-MCM-41 is the best systems for controlled and ordered release of L-Arginine.

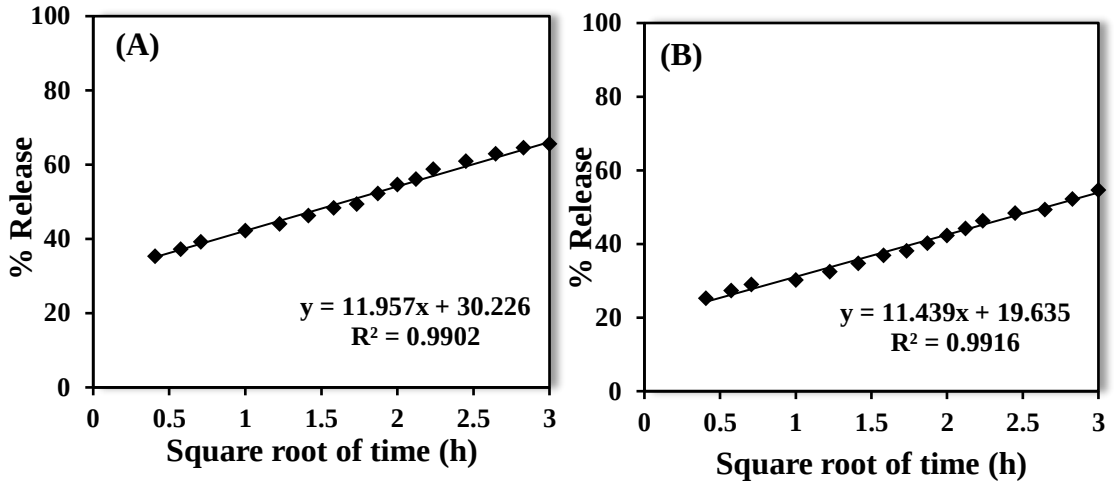

Figure 7. Higuchi Model for (A) L-arg/MCM-41 and (B) L-arg/TPA-MCM-41

Table 1. 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 R_1 = 0.975	K_1 = 0.0234 R_1 = 0.987
Higuchi model	K_2 = 11.95 R_2 = 0.990	K_2 = 11.43 R_2 = 0.991

In vitro controlled release of Cysteine from MCM-41 and TPA-MCM-41

(a) Preparation of Calibration curve for Cysteine

Stock solution of Cysteine was prepared by dissolving 1 g of it in 100 ml distilled SBF (10 mg/mL). Series of Nessler's tubes containing 0.2 to 0.8 mL of this stock solution was diluted up to the 4 mL using SBF. 1 mL of acetate buffer (pH 5.5) and 1 mL ninhydrin reagent (10 % ninhydrin solution in ethanol) were added to these solutions. All the tubes were put in boiling water bath up to 10-15 min for completion of reaction which was seen by the formation of purple colour. The solutions were cooled under tap water and then 1 mL of 50 % ethanol was added to each of the solutions. Absorption was taken at 570 nm wavelength using Perkin UV-Visible spectrophotometer and result is shown in Figure 8.

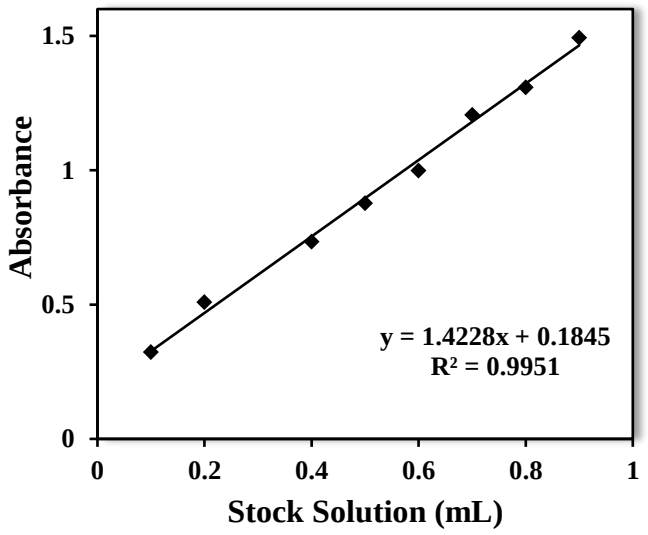

Figure 8. Calibration curve for Cysteine

(b) In vitro controlled release of Cysteine

The release profile of Cysteine was also obtained by same method which was used for L-Arginine. Cysteine loaded materials were soaked in 150 mL of a SBF (1 mg of the Cysteine sample per mL of fluid). This release fluid was analyzed for Cysteine content by treating it with 10 % ninhydrin solution at 570 nm. 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 9. Initially 38% and 60% of Cysteine is released in case of Cys-MCM-41 and Cys-MCM-41(I) respectively. Further slow and delayed release is observed for Cys-MCM-41. It is expected that in case of incipient wet impregnation method, 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). Also, controlled release in the case of Cys-MCM-41 indicates presence of relatively firmly bound Cysteine molecules inside the channels of MCM-41 during the soaking method. In vitro release study of Cysteine shows that more controlled release rate for Cys-MCM-41.

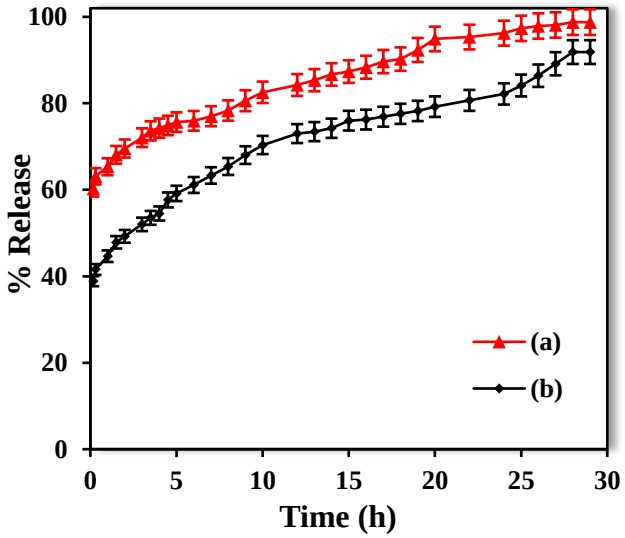

Figure 9. In vitro release profile of (a) Cys-MCM-41(I) and (b) Cys-MCM-41

(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 10. Both the systems, Cys-MCM-41 (Figure 10A) and Cys-TPA-MCM-41 (Figure 10B) show slower release under static condition. In case of MCM-41-Cys, initially 30% of Cysteine is released under static condition while 38% is released

under stirring condition. After that controlled release of Cysteine is observed and reached to 80% and 91% in 30 h under static and stirring condition respectively.

Similar observation is obtained for Cys-TPA-MCM-41. Initially, 28% and 34% 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 10B). The observed slower release of drug under static condition is may be due to the slower diffusion of drug which eventually decreases the dissolution rate of Cysteine from materials to the release medium and thus requires high time for complete release of Cysteine. Here more convenient result is obtained under stirring condition. So, further release profile of these materials was compared.

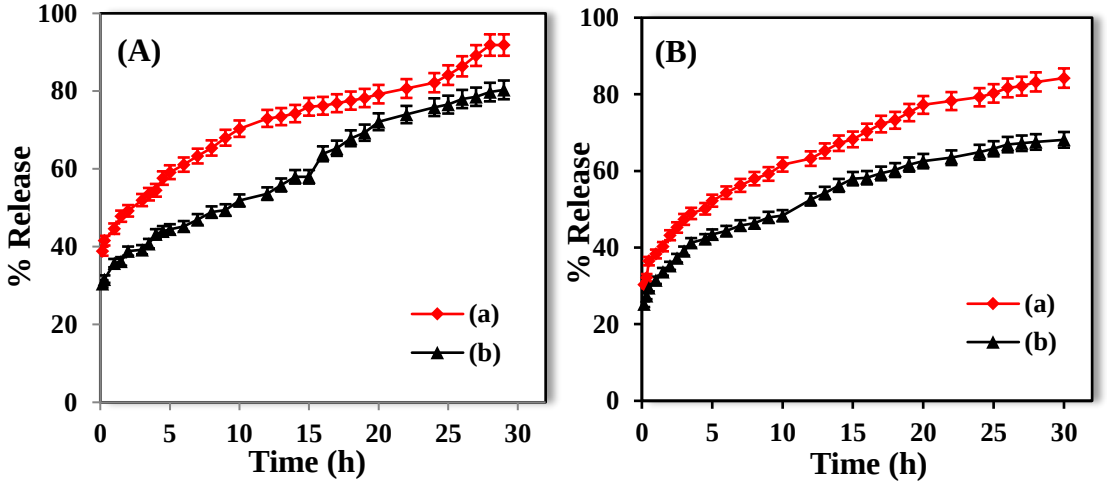

Figure 10. In vitro release profile of **(A)** Cys-MCM-41 and **(B)** Cys-TPA-MCM-41 under (a) stirring and static (b) condition

To see the effect of pH on release rate of Cysteine, release study was also carried out at lower pH. However, we did not get any release of Cysteine under acidic condition.

It is well known that in acidic condition cysteine remain as neutral species and all the functional groups C=O, NH2 and SH are free.

In Chapter 1A (FTIR, Figure), we have confirm that cysteine can bind through its functional groups to the surface Si-OH group of MCM-41. Therefore, in acidic condition it may bind through all the 3 groups showing strong interaction between them as a result Cys does not release under acidic condition.

On the other hand, at physiological pH (7.4), carboxylate and SH group deprotonated and interaction between Cys and carrier becomes comparatively weaker. Hence, under this condition Cys easily gets detached from carrier and release is observed.

(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-41 and Cys-TPA-MCM-41 were compared and results are shown in Figure 11. Initially, 38% and 30% of Cys is released and reached to 72% and 61% up to 10 h from MCM-41 and TPA-MCM-41, respectively. It reached to 91% and 84% up to 30 h for MCM-41 and TPA-MCM-41, respectively. Here, slower release profile is obtained for Cys-TPA-MCM-41 as compared to Cys-MCM-41. This is may be because of the more attractive interaction between the Cys molecules and TPA-MCM-41. 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 Cys from TPA-MCM-41.

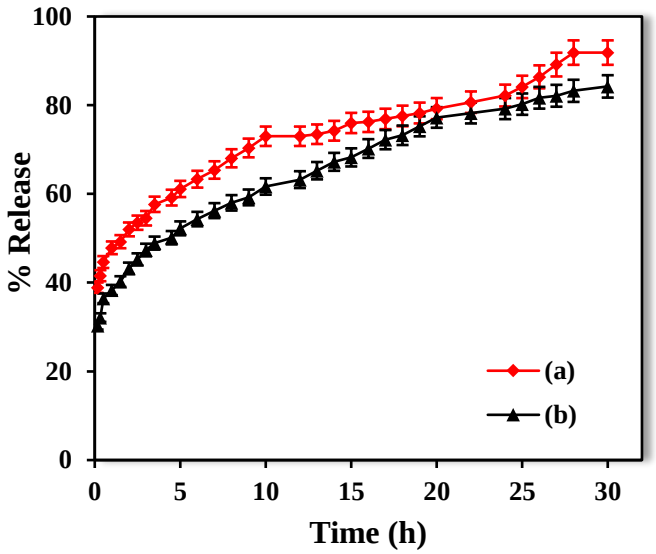

Figure 11. Comparison of release profile of (a) Cys-MCM-41 and (b) Cys-TPA-MCM-41

FTIR of Cys/TPA-MCM-41, after release study was also carried out for finding that the TPA is truly act as functionalizing agent and spectrum is shown in Figure 12. Spectrum is similar to that of TPA-MCM-41 and show entire bands related to TPA-MCM-41. Further, absence of any splitting in band of P-O bond suggests the intact structure of TPA-MCM-41. Along with this, bands corresponding to Cysteine are disappear which is due to the removal of it during release study.

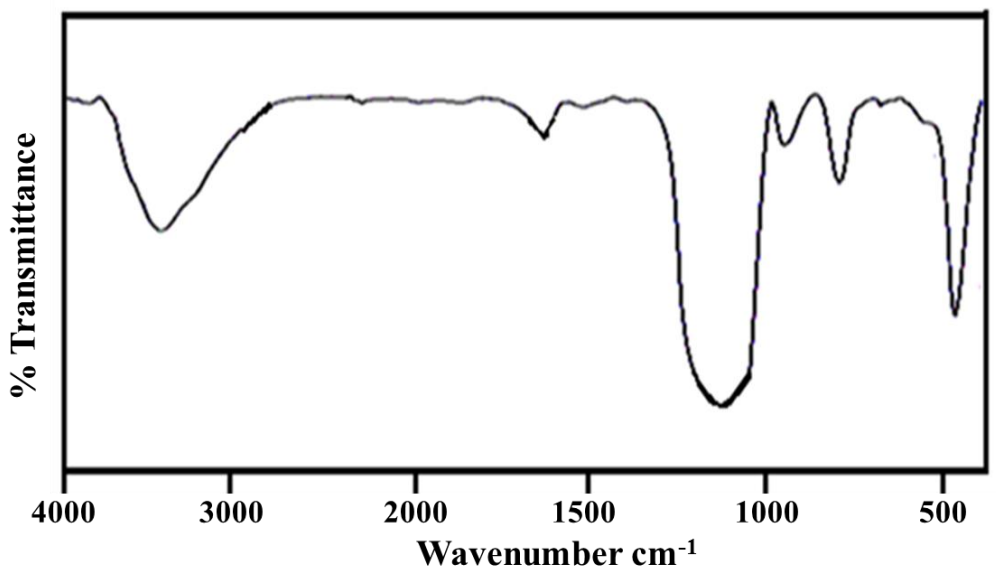

Figure 12. FTIR of Cys/TPA-MCM-41 after release study

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 as well as Higuchi model and kinetic parameters are shown in Table 2. Figure 13 shows the first order release kinetic model of Cys-MCM-41, Cys-MCM-41(I) and Cys-TPA-MCM-41 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-41 (0.9885) as compared to Cys-MCM-41 (0.9864) and Cys-MCM-41(I) (0.9361).

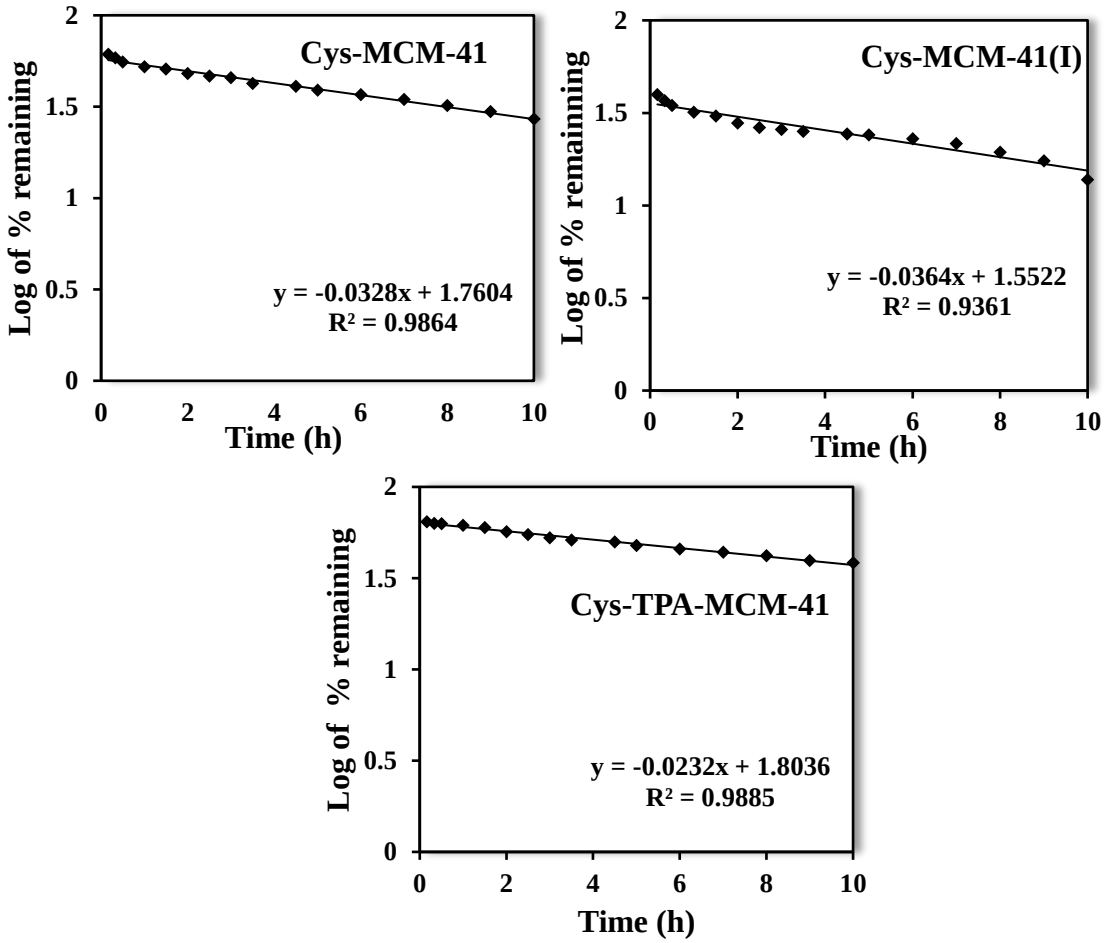

Figure 13. First order release kinetic model of Cys-MCM-41, Cys-MCM-41(I) and Cys-TPA-MCM-41

(b) 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 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-41(0.9959) compared to Cys-MCM-41 (0.9955) and Cys-MCM-41(I) (0.963).

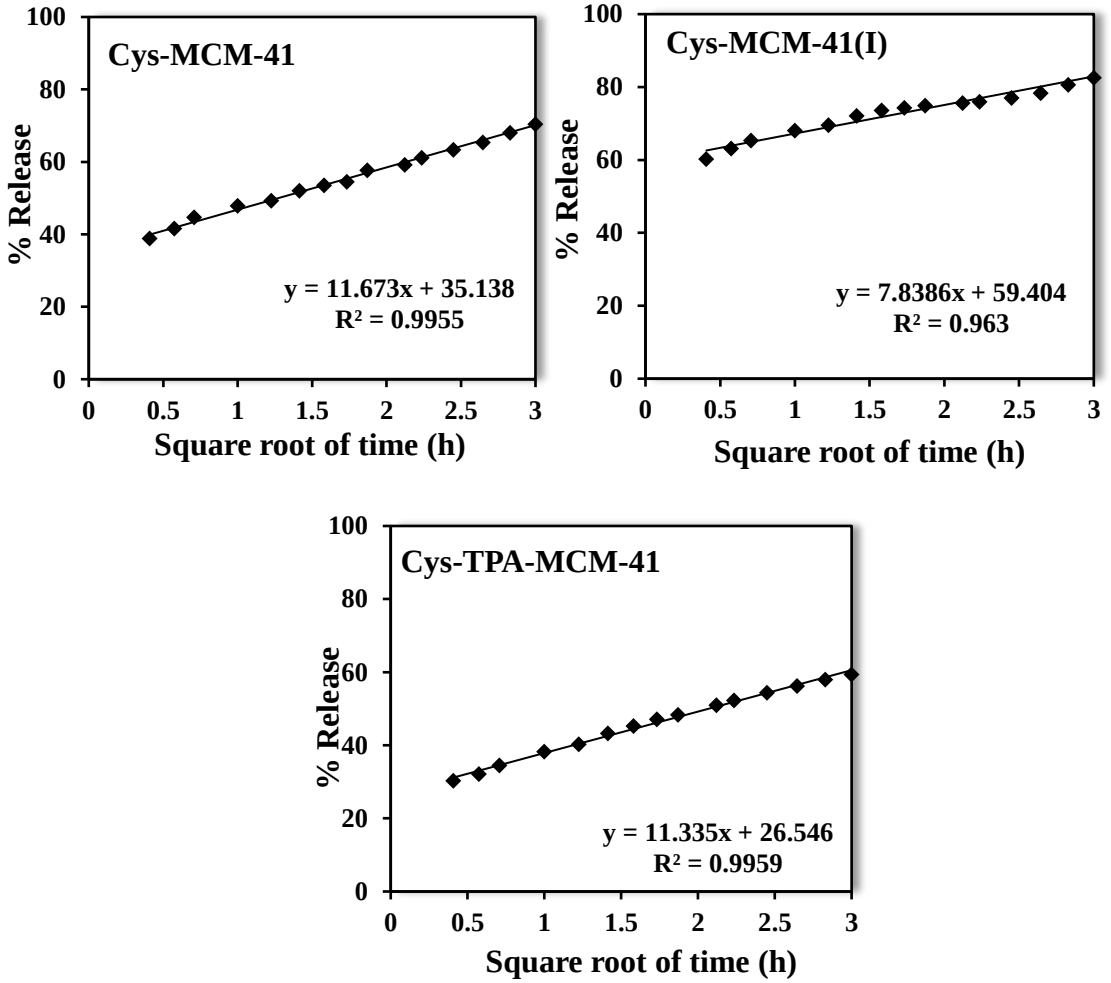

Figure 14. Higuchi model of Cys-MCM-41, Cys-MCM-41(I) and Cys-TPA-MCM-41

Here, more ordered release data is obtained for Cys-TPA-MCM-41 (Table 1). Thus, Cysteine release from these systems is concentration dependent process and follows first ordered release kinetics. Further it follows the Fickian diffusion mechanism as value of rate constant n is obtained up to 0.43 (Table 2).

Table 2. 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	$K_1 = 0.0363$ $R_1 = 0.936$	$K_1 = 0.0328$ $R_1 = 0.986$	$K_1 = 0.0232$ $R_1 = 0.988$
Higuchi model	$K_2 = 7.83$ $R_2 = 0.963$	$K_2 = 11.67$ $R_2 = 0.9955$	$K_2 = 11.353$ $R_2 = 0.9959$

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, slow release of L-Arginine was observed at lower pH as it shows more interaction with carrier.
- To see the effect of loading method on release rate, release profile of Cysteine loaded by different methods (wet impregnation and soaking) have been compared and result shows that more ordered and slow release was obtained for system which was prepared by soaking method.
- TPA has great influence on release rate as it has terminal oxygen through which it binds with L-Arginine/Cysteine and holds it for longer period of time and shows slower release.
- FTIR after release study, for both system (L-arg/TPA-MCM-41 and Cys/TPA-MCM-41) suggests that TPA truly acts as functionalizing agent only.
- Kinetic and mechanism study shows that release of L-Arginine/Cysteine follows first order kinetic and diffusion mechanism.

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