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RESEARCH ARTICLE

In vitro release kinetics study of Diallyl Disulphide entrapped into mesoporous silica matrix & evaluation of its antimicrobial activity

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ABSTRACT

Diallyl disulphife (DADS) is one of the major constituents of garlic which have antimicrobial activity as well as many other advantages for human health. Sulpher present in DADS is main the reactive one to deal with the microbes. Mesoporous silica nanomaterial (MSNs) is evaluated as potential drug carrier for any organic drug molecule to keep it intact for in vitro sustained release into body fluids. Comparing between two different body fluids, dissolution rate of DADS is more in SBF (pH 7.4) than SGF (pH 1.2) because of its acidic nature. In two medium the release mechanism is super case II transport as in both cases the 'n' value of koresmeyer peppas model is greater than 0.89 obtained through kinetic study. MIC value of DADS against Salmonella typhi is 0.941 mg/ml. Besides this microscopic analysis confirm the deformation of microbial cells. These means mesoporous silica nanomaterial entrapped with DADS and subsequently released in pH 7.4 is much more concerned with high amount of drug availability in body fluids.

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INTRODUCTION

Phytochemicals present in herbs is an important aspect now a day due to its lethal property on microorganisms as well as less side effects comparing to chemically synthesized drug. There is a better future of herbal medicine if it blends with nanotechnology. Nanomaterials have the capability to invade all the biological membrane present in our body. If phytochemicals are being entrapped within any nanomatrix then it will be very trouble-free for the particular herbal values to reach to any part of the body as undamaged condition [1]. Mesoporous silica nanomaterial (MSNs) has attracted much interest as a carrier of holding drug. The physical properties of silica nanomaterial (like shape, size, porosity, mesostructure etc), chemical natures (like surface chemistry, surface funtionalization, electric charge etc) can be mold as per experimental needs that is why silica based materials is very welcoming in biological world. Inorganic porous silica materials are durable drug carriers compared to organic polymers because it can protect the loaded drug from any chemical or biological degradation efficiently. Silica materials also have the resistant power to *in vivo* microenvironment's interruption like changes in ion concentration, pH, and sometimes temperature, pressure etc as it has extremely high thermal stability and chemical inertness than other polymers. Mesoporous silica nanoparticles have large surface area, multifunctional properties like integrating drug carrier, success in diagnostic and curative purpose, act as biocatalyst/ biosensing agent and thus it become more and more useful in biological field [2].

Literature says in contrast with allopathic drug, plant based natural yields are paying special attention in chemo-prevention because they have less or no side effect. It has been noticed that

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naturally occuring phytochemicals like diallyl disulphide (DADS) have many health beneficial effect. DADS is one of the main constituents of garlic (*Allium sativum*) as well as in onions (*Allium cepa*) and it is also the major constituent of secondary metabolites [3].

There are almost 700 species widely exist in the world of genus Allium [4]. The species are dissimilar in taste and morphology but they are appreciated because of their easy growth, flavor, long storage time and obviously their nearly similar phytochemical and biochemical property. Main constituent of these Allium species are organosulpher compounds which is the causative agents for organoleptic parameters [5]. These volatile constituents are the main parts of the essential oils which have antioxidant and anti microbial properties [6-11].

Alliin is the primary from of organosulfer compound which is transferred into allicin through enzyme allinase after cutting of garlic. Allicin is also an unstable compound and it immediately breaks down into its oil soluble derivatives (essential oil) which include diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), vinyl dithiin and ajoene under their respective favorable conditions [12].

Banerjee et. al. & Kim et. al. have reported through GC-MS analysis that more than 94.63% constituent of garlic essential oil is organosulfer compounds. These organo sulfer compounds include diallyl disulfide (37.90%), diallyl trisulfide (28.06%), allyl methyl trisulfide (7.26%), diallyl sulfide (6.59%), diallyl tetrasulfide (4.14%) and allyl methyl disulfide (3.69%) [13]. These diallylsulfides show antimicrobial activity as well as antioxidant property because of greater number of sulfer atoms present in it. So, the richness of sulfer atoms in garlic essential oil makes it more effective to exert above mentioned properties [6,14,15,16]. Among all the essential oils, diallyl disulfide (DADS) and diallyl trisulphide (DATS) are comparatively stable compounds. From the previous studies it has been observed that drug metabolism and pharmacokinetics of organosulfer compounds from garlic have been generally restricted on rodents. Literature gives us the information of metabolism and pharmacokinetics data mainly about DADS among all the organosulfer components of garlic [12].

Moreover, sulfide atoms do react with -SH groups of cellular proteins and generate mixed disulfides resulting damage of microbials cells is reported by Mynaer et al 2014 [13].Different carriers for example liposomes, nanoparticles, microemulsion have been developed for implementation of sustained released of drug which actually enhances the bioavailability, systemic circulation and constancy rate of drug. Depending on the stable and active organosulfer components, a stable drug delivery system is prepared for in the development of potential pharmaceuticles. The released molecules are evaluated as biopharmaceuticles [12].

In this paper, proper entrapment of DADS in the mesoporous silica nanoparticles and the study of release kinetics from the carrier as well as evaluation through kinetic modeling along with antimicrobial property is done.

EXPERIMENTAL SECTION

Materials

Tetraethyl orthosilicate (TEOS , $C_8H_{20}O_4Si$), Ethanol (C_2H_5OH, 99% pure), Hydrochloric acid about 35% (HCL), Sodium chloride (NaCl), Sodium Bicarbonate ($NaHCO_3$), Potassium chloride (KCl), Di potassium hydrogen phosphate (K_2HPO_4.3H2O), Magnesium choride(MgCl2.6H2O), Calcium chloride (CaCl2), Sodium sulphate (Na_2SO_4), Tris(hydroxymethyl) aminomethane ($(CH_2OH)_3CNH_2$) were purchased from Merck and used without any further purification. Nutrient broth was purchased from Himedia. Ultrapure De ionized water was used as a solvent for chemical synthesis.

Sol gel synthesis of mesoporous silica nanopaticles (MSNs)

The sol-gel process involved the manufacture of inorganic matrices through the formation of a colloidal suspension (sol) and the gelation of the sol to form a wet gel, which, after spontaneous drying, formed dry gel called xerogel. In most sol-gel techniques water and low molecular weight alkoxysilanes were used, such as tetraethoxysilane (TEOS). TEOS used as silica precursor. The alkoxide hydrolyses with water forming silanols, with acid as catalyst.

The reactions were made by a simple hydrolysis and condensation reactions. The hydrolysis reaction which could be acid or base catalyzed, replaces alkaloid groups by hydroxyl groups.

Hydrolysis reaction ≡ Si-OR + H2O → ≡ Si-OH + ROH

Alcohol condensation ≡ Si-OH + RO-Si≡ → ≡Si-O-

Si ≡ + ROH
Water condensation ≡ Si-OH + HO-Si≡ → ≡Si-O-Si ≡ + H2O
In case of condensation reaction siloxane bonds are formed [17].

Extraction of herbs

For the extraction of herb 20 gm of *Allium sativum* (garlic) and 20 ml of ethanol (99%, Merck) were mixed and kept for 1 hour within an air tight bottle. Then that mixer was blended with a mixer grinder to extract the active component Diallyl disulphide (DADS). After that the blended material was filtered with whatman 41 filter paper through a vacuum filtration. The supernatant part was considered & stored at below 4˚C for further experiment.

Incorporation of drug into MSNs

To make DADS entrapped silica matrix, DADS was added during the synthesis of silica nanomaterial through sol-gel process. During synthesis in the 2nd beaker where ethanol and water were already mixed, DADS was added drop wise at slow rate with continuous stirring for at least 30 minutes. Make a transparent and homogenous solution of ethanol, DI water and DADS then mixed it into the 1st beaker very slowly under a continuous stirring where TEOS and EtOH were mixed previously. After completion of total mixing, resultant sol was left at room temperature for at least 10 days to get biocompatible silica material.

Characterization

Surface properties (surface area, pore diameter, pore volume) of the synthesized silica nanoparticles were examined through N_2 adsorption desroption isotherm at 77.350 K on a mechanical pore diameter, surface area analyzer (Quantachrome Novawin, version 11.03). Surface area was measured through BET (Brunauer- Emmett- Teller) analysis method. Before experiment the samples were degassed under vacuum at 100 ˚C for 2 hour according to the samples.

Particle size and mesoporous nature of silica gel were analysed by field-emission scanning electron microscopy (FESEM, ZEISS) equipped with an energy-dispersive X-ray spectrometer (EDX). Before analysis, the samples were coated with platinum for 120 s using automatic magnetron sputter coater (Auto sputter coater,).

X-ray diffraction (XRD) patterns were analyzed with Rigaku, Ultima IV, Japan. The phase of the silica gel samples with (herbal values specially Diallyl disulphide) and without entrapped drug were analyzed.

Fourier transform infrared spectra (FTIR Schimadzu, Model: prestige 22) of the silica gel with and without encapsulated drug were obtained in the range 4000–400 cm^{-1}.

At required time interval released DADS from silica matrix samples were collected and analysed by UV –Vis spectroscopy (Perkin Elmer, Lambda 35 UV-VIS spectrophotometer).

Antimicrobial assay (MIC) was done in Micronaut system (Merlin, Germany) and data collected through Multiskan EX (Thermo, Finland) spectrophotometer system.

In vitro drug release study

In vitro drug release study was done at 37˚ C in two different pH medium namely SBF (simulated body fluid, pH-7.4) and SGF (simulated gastrointestinal fluid, pH-1.2). DADS as drug had been encapsulated at an amount of 50% of the total volume (w/v ratio) of raw materials of silica nanomaterial. Drug was added during the preparation of nanomaterial to do proper entrapment of the drug and to make a homogeneous solid drug entrapped silica matrix, as any part of the dried silica matrix could be used in burst release (BR) and sustained release (SR) without any concentration diversity. In case of burst release, 3 ml sample each time was taken from the beaker at the predetermined intervals of 15 min, 30 min, 45 min, 1 hr,2 hr, 3 hr, 4hr, 5hr & 6 hr and each time 3 ml fresh SBF or SGF is replaced to the beaker. But in sustained release, 168 hours was the total time duration with 24 hour time interval. Here also 3 ml samples were taken and replaced with 3 ml fresh SBF or SGF to maintain the concentration of total volume and analyzed with UV Vis spectroscopy. To check the reproducibility, all the experiments were repeated three times and the mean value was considered.

In vitro antimicrobial assay

A severe disease causing microorganism was selected for the study i.e, *Salmonella typhi* responsible for typhoid disease. It is a gram-negative, rod shaped and non filamentous bacteria sometimes lethal for human being. The antimicrobial assay MIC study was done in flat bottomed 96 well plate where at first 100 µl nutrient

Table 1. BET analysis of the sample

Sl. No	Sample name	Surface area (m^2/g)
1	MSNs	442.600

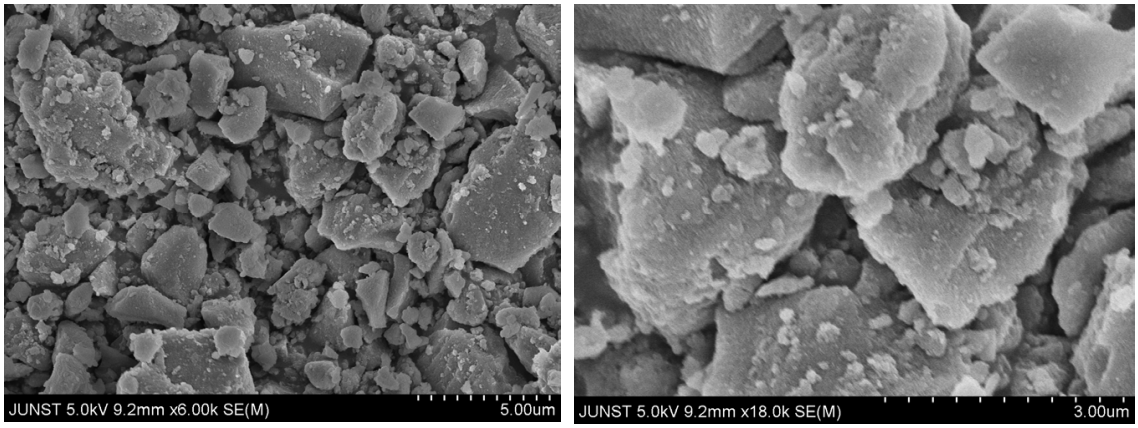

Fig. 1a&1b. Synthesized mesoporous silica nanoparticles samples in different magnifications

broth was added in each well. Then in the first column of the first row, 100 µl drug was added (i.e, in 12 well of first row) and take 100 µl from the 1st well to add the next well vertically. Similarly same procedure was followed for this column up to 8th well. The excess 100 µl from the last (8th) well of the last row was discarded to maintain the proper dilution. The same procedure was carried out for every column i.e, for rest of the 11 column. After mixing of nutrient and drug well, 10 µl of bacterial sample (0.5 McFaraland stabndard) was added to each well equally and take zero time optical density reading and after incubation (at 37˚C) 24 hour optical density reading at 620nm.

RESULTS & DISCUSSION

Characterization of Synthesized MSNs

BET Surface Area

Surface area was analyzed by BET method. As acid catalyst enhances weekly cross linking so, smaller particles were obtained compairing with base catalytic synthesis. Smaller particles meant of high surface area of mesoporous silica nanoparticles (Table 1) were considered to absorb more drugs and to release them easily in the medium.

FE-SEM analysis

Fig. 1a & 1b shows the SEM image of mesoporous silica nanoparticle samples in two different magnifications. The shape of silica gel is irregular in nature. These nanoparticles are very fine in nature and agglomerated with each other to form cluster.

XRD analysis

XRD analysis of bare silica nanoparticles & drug entrapped silica nanoparticles are represented in Fig 2. A hump between 15 to 30 in XRD graph is confirmed the amorphous nature of silica nanoparticles. Amorphous silica nanoparticles are non toxic to the human body and also biodegradable.

FTIR Analysis

Fig. 3 represented the FTIR spectra of bare mesoporous silica nanoparticles and DADS entrapped silica nanoparticles within the range 400-4000 cm^{-1} (Table 2). The frequency near 460cm^{-1} and 800 cm^{-1} i.e., 449 cm^{-1} & 803 cm^{-1} (Red line) and 460 cm^{-1} & 803 cm^{-1} (Black line) were observed for out of plane bending of Si-O-Si bond and the stretching vibration of Si-O bond respectively. In the 900-1300 cm^{-1} interval an almost complete overlap of the Si-alkoxy compounds with the siloxane bands was realized. The presence of very broad strong peak at 1083 cm^{-1} can be assigned as the stretching of C-O bond of TEOS and ethanol both. A strong absorption band between 3300 and 3500 cm^{1} assigned to O–H stretching in H-bonded was observed in both mesoporous bare and DADS entrapped silica for molecularly adsorbed water. Also this band can be cross-checked through the

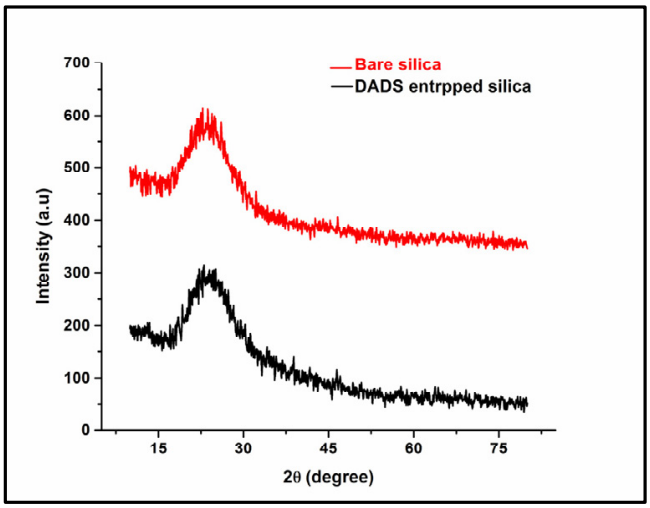

Fig. 2. XRD analysis of bare silica & drug entrapped silica

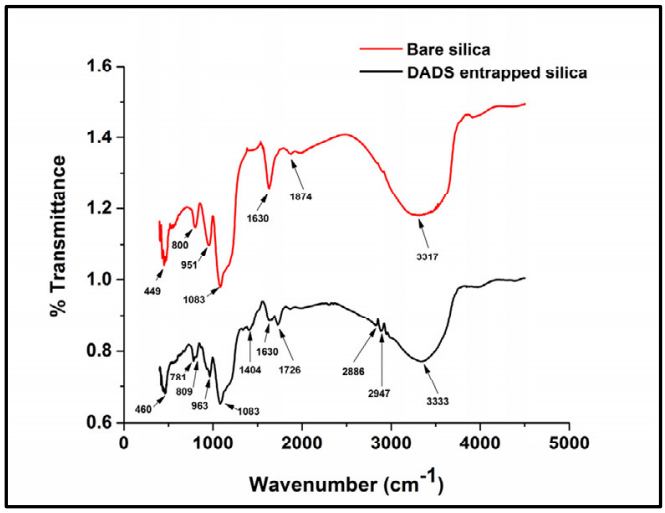

Fig. 3. FTIR graph of Bare silica nanoparticle & DADS entrapped silica nanoparticle

1630 cm^{-1} band due to scissor bending vibration of molecular water [18]. Compairing to bare silica, there were many new absorptions found in the spectrum of DADS entrapped silica matrix presented as black line. The absorption in 781cm^{-1} represented the bond between C-S-C which indicats the presence of Diallyl sulfide in the silica matrix.

From the literature it was found that 2870-2960 cm^{-1} vibration was for aliphatic C-H bond. Wave number 2886 cm^{-1} in Fig. 3 indicated that the presence of aliphatic C-H in DADS entrapped silica matrix. The stretching frequency of C-S bond was present in between 900-1100 cm^{-1} which implies that peak was present at 963 cm^{-1} due to the presence of Carbon Sulpher bond in the garlic entrapped silica matrix [19]. From literature it can be found out that 400-500 cm^{-1} represents S-S bond [20] and near 450 cm^{-1} was assigned for Si-O-Si bending [21]. In DADS entrapped silica matrix, vibration for Si-O-Si bond (449 cm^{-1}) was merged with vibration of S-S bond (400-500 cm^{-1}) and was red shifted to 460 cm^{-1} due to the entrapment of DADS in the silica.

From all the corresponding FTIR data, it could be concluded that DADS (extracted) were entrapped within mesoporous silica nanomatrix and most importantly it do not form any chemical bond with

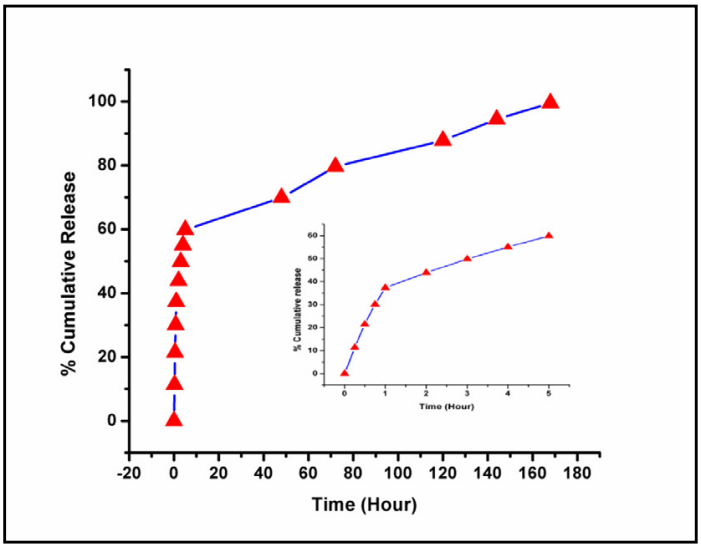

Fig. 4. *In vitro* burst and sustained release profile of DADS in SBF medium

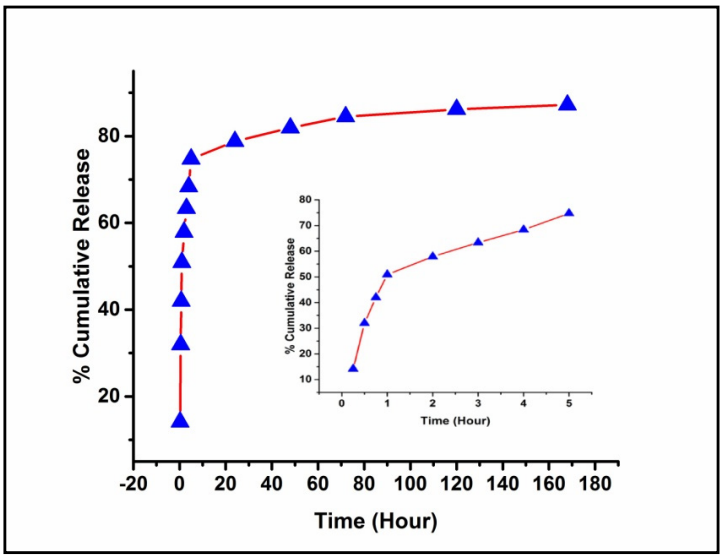

Fig. 5. *In vitro* burst and sustained release profile of DADS in SGF medium

its matrix because there was no obvious changes in FTIR peaks entrapped silica nanoparticles. So, in further experiments drug releases would be easy in any medium.

Dual drug release profile study

Extracted DADS was confirmed through UV-Vis spectroscopy analysis (Absorbance 210 nm) [22]. Fig. 4 and Fig. 5 had shown the biphasic release profile of DADS against time of drug release (0 to 168 hour) in SBF medium having pH 7.4 (blue line) and in SGF medium having pH 1.2 (red line) respectively. Burst release profile of each was also illustrated in the respective graph.

Comparing the release profile (from Fig. 4 & Fig. 5) of drug in SBF & SGF both it was observed that drug dissolution is more in SBF medium than in SGF. Burst released and sustained released samples were examined and the optical values (taken at 210nm) considered making liquid liquid kinetics specially fitted on First order, Zero order and Higuchi model. The best fitting model (i.e R^2 values towards 1) described the process of dissolution. And lastly fitted on the korsmeyer peppas model

Table 2. FTIR study bare & DADS entrapped mesoporous silica nanoparticles

Wave Number (cm^{-1})	Vibration Type	References
400-500 cm^{-1}	S-S stretching	C.N.R. Rao et al 1963
Near 460 cm^{-1} & Near 803 cm^{-1}	Si-O-Si bending	R. K. Nariyal,2014
781 cm^{-1}	C-S-C stretching	B. Tasci et al 2016
900-1100 cm^{-1}	C-S stretching	B. Tasci et al 2016
900-1300 cm^{-1}	C-O stretching	Pilinio 2003
1630 cm^{-1}	H-O-H bending	Pilinio 2003
2870-2960 cm^{-1}	C-H stretching	C.V Moraru et al, 2015
3300 cm^{-1} - 3500 cm^{-1}	O-H stretching	C.V Moraru et al, 2015

Table 3. Kinetic values obtained from 0 to 168 hours of release at pH 7.4

Sample name pH-7.4		Zero order (R^2 value)	First order (R^2 value)	Higuchi model (R^2 value)	Korsmeyer-peppas (n value)
MSNs	BR	0.821	0.927	0.960	1.82
	SR	0.983	0.771	0.804	3.66

Table 4. Kinetic values obtained from 0 to 168 hours of release at pH 1.2

Sample name pH-1.2		Zero order (R^2 value)	First order (R^2 value)	Higuchi model (R^2 value)	Korsmeyer-peppas (n value)
MSNs	BR	0.794	0.913	0.923	1.81
	SR	0.912	0.920	0.960	1.41

Table 5. MIC data of *Salmonella typhi*

Sl no	Microorganism used	Concentration(mg/ml)
1	Salmonella typhi	0.941

where the 'n' value was used to find out the drug release mechanism. For the case of cylindrical tablets, 0.45 ≤ n corresponds to a Fickian diffusion mechanism. If 'n' value lies between 0.45 to 0.89 that means it is non-Fickian transport and if n = 0.89 then it is Case II (relaxational) transport, and when n is greater than 0.89 then it is super case II transport [23].

Table 3 and Table 4 contained the regression coefficient (R^2) values of above mentioned kinetic models. Towards 1 R^2 values were considered as the best fitted for this set of study.

In 7.4 pH medium Burst release followed Higuchi model and SR followed Zero order model (Table 3) and in 1.2 pH Burst release as well as SR both followed Higuchi model (Table 4).

As the release follow higuchi model that means initial concentration drug in the subsequent matrix is much higher than the soluble drug concentration in the dissolution medium. The smaller drug particles were discharged in one dimension through diffusion. This model also states that total exhaustion of drug is not possible or cannot be achieved during dissolution. In both SBF & SGF plus in both releases 'n' value of koresmeyer peppas model was greater than 0.89 that means all the release mechanism is super case II transport.

MIC study

Minimum inhibitory concentration was inversely proportional to the growth of the respective bacteria. Microbial growth or presence of living microbes could be examined through optical density measurement. 96 ELISA flat bottomed plates were considered to do the experiment. MIC value was 0.941mg/ml (Table 5). As in the graph

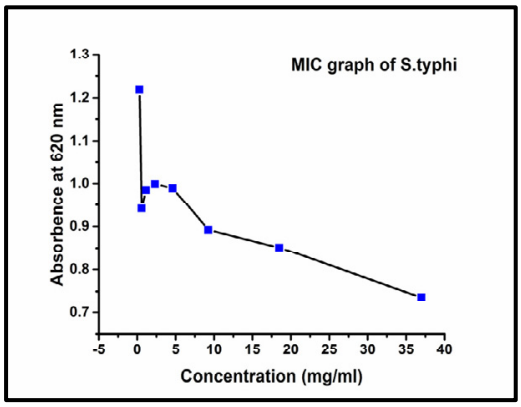

Fig. 6. MIC graph of *Salmonella typhi*

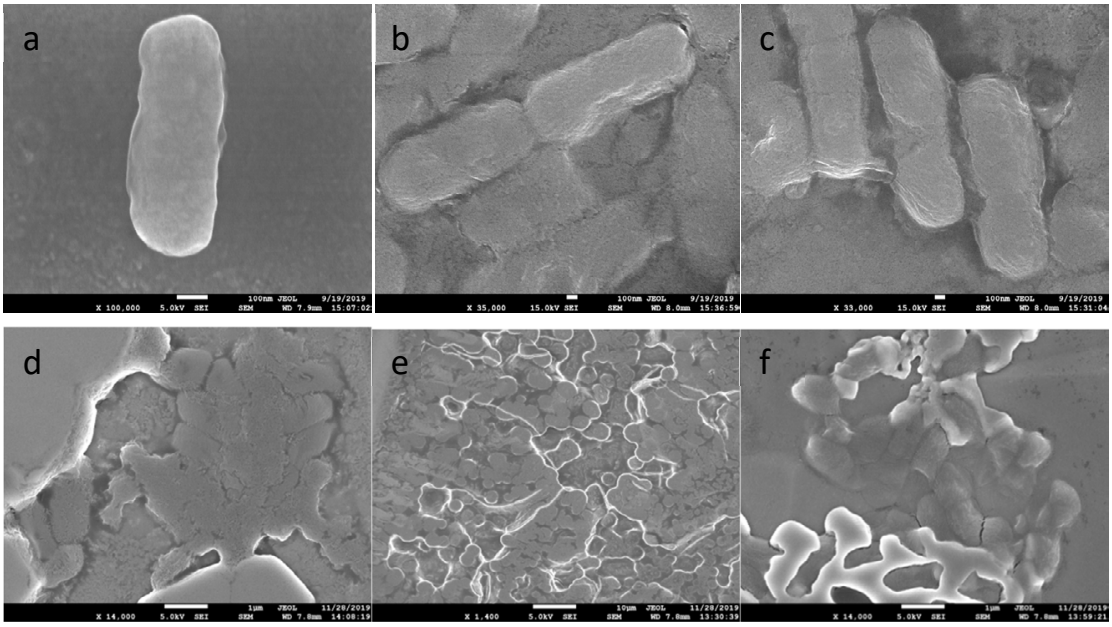

Fig. 7. FESEM images of *S. typhi :* a,b,c & d,e,f are the picture of microorganism before treatment and after treatment respectively.

shown in Fig. 6, there was a sharp fall of bacterial growth at this point, after that as the concentration of the drug was increased the bacterial growth gradually decreased.

Microscopic analysis of Microorganisms

FESEM images of *S. typhi* were shown in Fig. 7. Microscopic analysis revealed the structure of bacteria treated with sustained released drug from drug entrapped nano carrier. Here the cell wall of all the bacteria was deformed; they became smaller in size, mostly fused together and appeared almost like the cell wall deficient L forms. The drug i.e, garlic especially the active component diallyl disulphide could damage the cell structure and function as already reported.

CONCLUSION

DADS entrapped mesoporous silica nanoparticles was synthesized through sol gel technique at room temperature and studied *in vitro* release kinetics in both simulated body fluid & simulated gastrointestinal fluid successfully. In this biphasic release study both burst and sustained release phase were best fitted to higuchi model for simulated gastrointestinal fluid but it is found that for simulated body fluid burst release followed to higuchi model whereas sustained release

was matched to zero order model. Comparing the release profile of two different medium it is observed that cumulative release percentage is higher in simulated body fluid of pH 7.4 as DADS could dissolute at pH 8.5 or below because DADS is slightly acidic in nature that means in higher pH it can release H^+ ions and stay at ionic configuration subsequently make it soluble in the medium. In all cases the mechanism of drug release indicating super case II transport as 'n' value was greater than 0.89 of koresmeyer peppas model. Minimum inhibitory concentration of released DADS from mesoporous silica nanoparticles reveals that it can control the growth of *S. typhi* microorganism.

COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of potential conflicts of interest

The authors declare that they have no conflicts of interest

Research involving Human Participants and/or Animals

No

Informed consent

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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