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Article

Role of drug adsorption onto the silica surface in drug release from mesoporous silica systems

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TITLE PAGE

TITLE

Role of drug adsorption onto the silica surface in drug release from mesoporous silica systems

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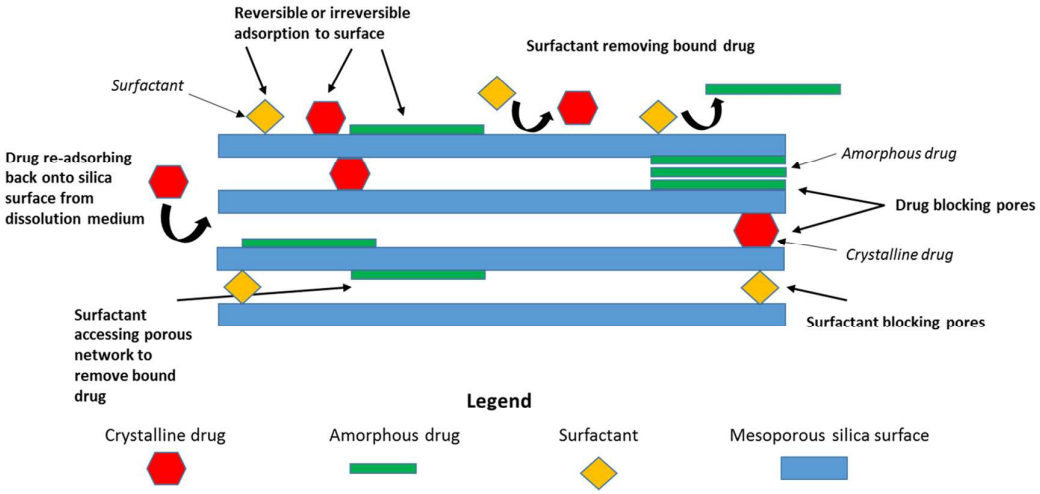
GRAPHICAL ABSTRACT



ABSTRACT

Factors contributing to incomplete drug release from a number of mesoporous silica formulations are not well understood. This study aims to address this gap in knowledge by exploring the role of drug adsorption onto silica substrates during the drug release process in dissolution media. Adsorption isotherms were generated to understand drug adsorption behaviour onto the silica surface. Two silica materials were selected (SBA-15 (mesoporous) and Aerosil®200 (non-porous)) to investigate the influence of porous architecture on the adsorption/dissolution processes. The ability of the dissolution medium to wet the silica surface, particularly the porous network, was investigated by the addition of a surfactant to the dissolution medium. The results demonstrated that a larger amount of drug was bound/m^2 to the non-porous surface than to the mesoporous material. Adsorption isotherms proved useful in understanding drug adsorption/release behaviour for the non-porous silica formulation. However, the quantity of drug remaining on the mesoporous silica surface after dissolution was significantly higher than the amount predicted using adsorption isotherm data. These results suggest that a fraction of loaded drug molecules were tightly bound to the silica surface or attached to sites which are inaccessible for the dissolution media. The presence of surfactant, sodium dodecyl sulphate, in the media enhanced drug release from the silica surface. This behaviour can be attributed to both the improved wetting characteristics of the media and adsorption of the surfactant to the silica surface. The findings of this study reinforce the significance of the role that silica porous architecture plays in the dissolution process and indicates that accessible surface area is an important parameter to consider for mesoporous systems in relation to drug release.

KEYWORDS

Adsorption; isotherm; dissolution; mesoporous silica; surfactant; porous architecture

1. INTRODUCTION

Loading drugs onto mesoporous silica materials has been considered as a formulation strategy to improve the aqueous solubility of BCS Class II drugs [1-3]. The high surface area and large pore volume of these silica carriers render them attractive substrates for enhancing drug dissolution [4]. Drug molecules loaded onto the silica surface exist in a stabilised amorphous state which greatly enhances drug solubility and dissolution rate [5-7]. In recent years, research in this area has focused on the development of various drug loading methods for these carriers [8-10] and the first *in vivo* animal studies have been conducted [11-13]. However, there remains a gap in knowledge as regards understanding the mechanism of drug dissolution from mesoporous silica formulations [3]. Incomplete *in vitro* drug release from these systems has been reported by many groups in the literature [9, 14, 15]. However, the factors contributing to these observations in dissolution experiments, performed under sink conditions, are not well understood. A study by Bui *et al* explored the use of mesoporous silica materials as adsorbents for chemicals found in pharmaceutical wastewater [16]. They determined that some drug molecules could bind irreversibly onto the silica surface. However, the impact of irreversible drug binding on the release of drug from mesoporous silica formulations has not been considered in the literature to date.

The aim of this study was to elucidate the role of drug adsorption onto porous and non-porous silica substrates, during drug release from these systems. Adsorption isotherms were generated to understand drug adsorption onto the silica surface. This equilibrium process describing drug bound to the silica surface and drug existing in solution emerged as a significant factor in gentamicin release from a silica carrier in a study by Xue *et al* [17]. In this work, sulphamethazine (SZ) was chosen as the model drug. Sulphamethazine has the potential to form amine-hydroxyl hydrogen bonds with the silica surface [17]. Two silica substrates were selected (SBA-15 (mesoporous) and Aerosil®200 (non-porous)) to investigate

the influence of porous architecture on the adsorption process. The extent of passive drug adsorption was quantified and compared with drug retained during dissolution experiments to determine whether isotherms can predict the extent of drug release from these formulations.

The ability of the dissolution medium to influence drug adsorption and release was considered through the addition of a surfactant to the dissolution medium. Sodium dodecyl sulphate (SDS), an anionic surfactant, was chosen as it is a common excipient added to dissolution media and formulations to improve the wetting characteristics and the solubilisation of drug molecules [18]. Sulphamethazine dissolution from SZ/silica systems in 0.1M HCl media was compared with drug dissolution in media containing surfactant to determine if improved dissolution media wetting capability enhances drug release from silica systems.

2. EXPERIMENTAL SECTION

2.1. Materials

SBA-15 was obtained from Glantreo Ltd. (Ireland). Aerosil®200 Pharma was sourced from Evonik Industries (Germany). Silica surface and pore properties were obtained from suppliers (Table 1). Sulphamethazine (SZ) and sodium dodecyl sulphate (SDS) (>98.5%) were purchased from Sigma Aldrich (Ireland). Liquid carbon dioxide was supplied by Irish Oxygen Ltd (Ireland). All other chemicals and solvents were of analytical grade or HPLC grade and purchased from Sigma-Aldrich (Ireland).

Table 1. Properties of silica materials obtained from suppliers

Silica Material	*Porosity*	*Particle Size (µm)*	*Surface Area (m^2)*	*Pore Volume (cm^3)*	*Pore Diameter (Å)*
SBA-15	Mesoporous	30	678.57 ± 8.23	0.64 ± 0.02	51.85 ± 0.05
Aerosil®200	Non-porous	12	200.00 ± 25.00	N/A	N/A

2.2. Surface Tension Measurements

Surface tension was determined experimentally using a KRUSS processor tensiometer K12 (KRUSS GmbH, Germany) with a platinum Wilhelmy plate. The plate was washed with deionised water, followed by an ethanol wash and subsequently flamed over a Bunsen burner after each measurement. All measurements were performed at 37 ^{o}C which was maintained with the HAAKE water bath (Thermo Fisher Scientific Inc., USA). Full independent replicates were performed in triplicate. Critical micellar concentrations (CMC) of SDS in deionised water and 0.1M HCl were determined by analysing changes in surface tension over the surfactant concentration range investigated.

2.3. Solubility Measurements

Solubility studies were performed in triplicate by the addition of excess sulphamethazine (SZ) to 10 ml of buffer media (0.1M HCl) using a standardised shake-flask method with a total shaking time of 48 h at 37^{o}C. Samples (2 ml volume) were removed at 24 h and 48 h time points and centrifuged at 16,500*g* for 13 min using a Hermle z233M-2 fixed angle rotor centrifuge, (HERMLE Labortechnik GmbH, Germany). The supernatant was removed and centrifuged again under the same conditions. The resultant supernatant was analysed using HPLC following dilution with mobile phase.

2.4. Adsorption Studies

Sulphamethazine adsorption studies were performed in screw-capped glass vials containing 100 mg of silica (SBA-15 or Aerosil®200) in 20 ml of SZ solution at a defined concentration in buffer (0.1 M HCl, pH 1.2). Experiments were conducted under the same conditions as solubility measurements i.e. shake-flask conditions for 24 h at 37 ^{o}C. At 24 h, samples (2 ml) volume were removed and centrifuged at 16,500 *g* for 13 min using a Hermle z233M-2 fixed angle rotor centrifuge, (HERMLE Labortechnik GmbH, Germany). The supernatant was removed and centrifuged again under the same conditions. The resultant supernatant was analysed using HPLC following dilution with mobile phase.

Adsorption studies were also conducted under the same conditions in the presence of a surfactant (sodium dodecyl sulphate (SDS)) at two defined concentrations, 10 mM SDS and 50 mM SDS. These concentrations were chosen as they reflect the range of concentrations approved for SDS by the U.S. Food and Drug Administration (FDA) Dissolution Methods [19]. An isotherm was generated by plotting the concentration of drug (mM) in solution at 24 h (x-axis) versus the quantity of drug adsorbed (mmol) per gram or per m^2 of the silica carrier (y-

axis). Linearised forms of the Langmuir [20] and Freundlich [21] isotherms were applied to the experimental data and the parameters determined are detailed in Table 2.

Table 2. Linearized forms of Langmuir and Freundlich Isotherms

Name	Linearised Form	Plot	Parameters
Langmuir	$F/B = (1/a.Nt) + F/Nt$	F *versus* F/B	a = slope/intercept N_t = 1/slope
Freundlich	$mlogF = logB + a$	logF *versus* log B	a = intercept m = slope

where B is the concentration of drug adsorbed to the silica surface, F is the concentration of free substrate in solution at 24 h, N_t is the total number of binding sites and a is related to the average binding affinity

2.5. Preparation of Sulphamethazine Loaded Silica Formulations

Sulphamethazine loaded silica formulations were prepared according to the method previously described by Ahern et al [15]. The drug and silica material was combined at a ratio of 1 mg SZ: 3 m^2 silica (SBA-15 or Aerosil®200) in a BC 316 high-pressure reactor (High Pressure Equipment Company, USA) and stirred using a magnetic stirring. The reactor was heated to 40 °C using heating tape and maintained at this temperature for the duration of the experiment. Temperature was monitored using a temperature monitor (Horst GmbH, Germany). The reactor cell was filled with liquid CO_2 and a high pressure pump (D Series Syringe Pump 260D, Teledyne ISCO, USA) was used to pump additional CO_2 to a final processing pressure (27.58 MPa). After 24 h, the cell was depressurised rapidly by venting the CO_2. The processed material was collected from the cell and stored in a desiccator prior to analysis.

2.6. Drug Content Quantification

The sulphamethazine content of the silica formulations were determined by thermogravimetric analysis (TGA), using a TGA 500 instrument (TA Instruments Ltd., United Kingdom). Samples in the weight range 2–10 mg were loaded onto tared platinum pans and heated from ambient temperature to 900 °C, at a heating rate of 10 °C/min under an inert N_2 atmosphere. Samples were analysed in triplicate. The drug quantity was calculated based on the weight loss between 100 and 900 °C, corrected for the weight loss over the same temperature range for a silica reference sample [14]. TGA thermograms were analysed using Universal Analysis 2000 software (TA Instruments Ltd., United Kingdom). Drug-loading efficiency was calculated using Equation 1:

$$Drug\ loading\ efficiency\ (\%) = \frac{Actual\ drug\ loading\ (mg)}{Theoretical\ drug\ loading\ (mg)} * 100 \quad \text{Equation 1}$$

The theoretical drug-loading was based on mass fraction of drug and silica used to prepare samples.

2.7. Dissolution Studies

Dissolution studies were performed in triplicate using USP II apparatus (Erweka® DT600 dissolution test system (ERWEKA GmbH, Germany)) in 500ml buffer (0.1M HCl, pH 1.2) at 37 ± 5^{o}C at a paddle rotation of 75 rpm. Sink conditions were employed for all dissolution experiments. A fixed mass of unprocessed drug (150 mg) or a mass of drug-silica formulation equivalent to 150 mg of drug was added to the dissolution medium. Samples of 4 ml volume were withdrawn at 1, 5, 10, 15, 30, 60 and 120 min intervals with an additional sample taken at the 24 h time point. Samples were immediately replaced with an equal volume of fresh, pre-warmed medium. The withdrawn samples were centrifuged at 16,500 *g* for 13 min using a Hermle z233M-2 fixed angle rotor centrifuge, (HERMLE Labortechnik GmbH, Germany).

The supernatant was removed and centrifuged again under the same conditions. The resultant supernatant was analysed using HPLC following dilution with mobile phase.

Dissolution studies were repeated as above with the addition of surfactant (SDS) to the dissolution media at two concentrations (10 mM and 50 mM).

2.8. HPLC Analysis of Sulphamethazine and Sodium Dodecyl Sulphate

Reversed phase high performance liquid chromatography (HPLC) was performed using an Agilent 1200 series HPLC system (Agilent Technologies, USA) equipped with both a Photo Diode Array Detector (DAD) and an Evaporative Light Scattering Detector (ELSD) in series. To quantify drug content in adsorption and dissolution studies without surfactant a reversed-phase column Kinetex C-18 column (150 mm × 4 mm) with internal pore width 2.6 μm (Phenomenex Ltd., United Kingdom) was utilised. An isocratic HPLC-DAD (diode array detector) technique adapted from a method by Ding et al [22] with a mobile phase consisting of acetonitrile – water – acetic acid (25:75:0.05), an injection volume of 50 μL and a flow rate of 1 $ml.min^{-1}$ at ambient temperature was employed. The detection wavelength was 265 nm. The retention time for sulphamethazine was 5.9 min.

To quantify both drug and surfactant concentrations in adsorption and dissolution studies, a HPLC-ELSD method adapted from Im *et al* [23] was utilised. The ELSD system was operated with an evaporative temperature of 80^{o}C, a nebulizer temperature of 70 ^{o}C and a N_2 gas flow rate of 1.0 $L.min^{-1}$. A reversed-phase column Prodigy ODS-3 column (150 mm × 4.6 mm) with internal pore width 5 μm (Phenomenex Ltd., United Kingdom) was utilised. Drug and surfactant were separated using a mobile phase gradient which consisted of two solutions: eluent A (water (25 mM ammonium acetate)) and B (acetonitrile). The gradient program started with 5% eluent B for 2 min, followed a 6 min gradient up to 95% eluent B. The column was then equilibrated with starting conditions for 2 min before the next injection. The

flow rate was 1ml.min^{-1} with an injection volume of 10 µL. Column temperature was set to 30^{o}C. The retention time for sulphamethazine and sodium dodecyl sulphate was 5.9 min and 7.4 min, respectively.

2.9. Pore size analysis of Mesoporous Silica Systems Before and After Dissolution

Pore size analysis by nitrogen (N_2) adsorption of the mesoporous sulphamethazine-SBA-15 formulation was carried out using a Gemini VI surface area and pore size analyser (Micromeritics, USA). Aerosil®200 is a non-porous silica material so porosity analysis was not undertaken. The samples were degassed overnight at 100 °C in a FlowPrep 060 sample degas system (Micromeritics, USA) prior to analysis. During analysis, liquid N_2 at −196 °C maintained isothermal conditions. The mesopore volume along with mesopore width were calculated using the Barrett–Joyner–Halenda (BJH) adsorption correlation [24]. Samples were analysed in duplicate.

2.10. Statistical Analysis

All statistical analyses were conducted using Microsoft Excel 2013 (Microsoft, USA) and GraphPad Prism (ver. 5, GraphPad Software Inc., USA). Results are expressed as mean ± standard deviation. *In vitro* dissolution and adsorption isotherm data comparing both formulations at different time points and concentrations respectively were tested for significance using a two-tailed, independent sample *t*-test, assuming Gaussian distribution and equal variance ($p < 0.05$ was considered significant).

3. RESULTS

3.1. Sulphamethazine (SZ) Loading Efficiency

Sulphamethazine was loaded onto both silica substrates at a theoretical ratio of 1 mg SZ/ 3 m^2 silica surface area. Sulphamethazine loading onto SBA-15 was 190 mg/g silica corresponding to a drug loading efficiency of 75.86% (calculated using Equation 1). SZ drug loading onto Aerosil®200 was 60 mg/g silica, equivalent to an 88.62% loading efficiency. These results are in line with loading efficiencies previously reported using SC-CO_2 methods [15].

3.2. Solubility Studies

SDS increases the solubility of some drugs above its CMC (critical micellar concentration) [25]. In this study, the CMC of SDS in both deionised water and 0.1M HCl were determined. The CMC of SDS in deionised water at 37^{o}C was 7.3 mM (0.21% w/v), while in 0.1 M HCl solution at 37^{o}C it was 0.8 mM (0.023%). Therefore, both concentrations of surfactant investigated in this study (0.3% w/v and 1.44% w/v) were above the CMC in 0.1M HCl. Drug solubility in each of the adsorption/dissolution media investigated are displayed in Table 4. SZ solubility in 0.1 M HCl and 0.1 M HCl with 10 mM SDS (0.3% w/v) were not significantly different. At the higher concentration of surfactant (50 mM SDS (1.44% w/v)), SZ solubility (38.80 ± 0.40 mM) was significantly higher than in the other two media ($p < 0.01$). However, SZ solubility enhancement in the presence of both concentrations of SDS was considered marginal.

3.3. Adsorption Studies

3.3.1. Sulphamethazine Adsorption onto Silica in 0.1 M HCl Medium

Adsorption isotherms for sulphamethazine adsorption onto SBA-15 and Aerosil®200, at the 24 h time point, in 0.1M HCl at 37^{o}C, are displayed in Figure 1 as both *mmol SZ/g silica* adsorbed and *mmol SZ/m^2 silica* adsorbed. Drug adsorption onto the Aerosil®200 non-porous

surface levelled off. In contrast, adsorption onto the mesoporous surface increased with increasing drug concentration. There was more drug bound per m^2 to the non-porous silica surface than the mesoporous SBA-15, indicating the drug cannot access the porous network in its entirety.

Adsorption data for the porous and non-porous silica systems were fitted to the Langmuir and the Freundlich adsorption models (Table 3). While both models were capable of describing the data for both silica substrates ($R^2 > 0.90$), the Langmuir model emerged as the best-fit for the adsorption of SZ onto non-porous Aerosil®200. In contrast, drug adsorption onto mesoporous SBA-15 was best described by the Freundlich model. The Langmuir model parameters were calculated for both silica substrates. The number of binding sites on the surface (N_t (mmol/m^2)) was determined to be greater for the non-porous Aerosil®200 than SBA-15, indicating that drug molecules cannot access the full extent of the SBA-15 porous architecture. The binding affinity (designated as a (mM)) of drug to the silica surface was equivalent for both the mesoporous material and the non-porous Aerosil®200 (*adsorbed SZ mmol/m^2 silica*). Freundlich model parameters were also calculated for both substrates and are displayed in Table 3. The heterogeneity index (m) is defined over a range of 0 to 1 with values closer to 1 describing a more homogenous system. The non-porous material exhibited the most homogenous surface of the two materials. The Freundlich equation binding affinity parameter (K_o (mM)), revealed stronger binding affinities between the drug and the non-porous surface.

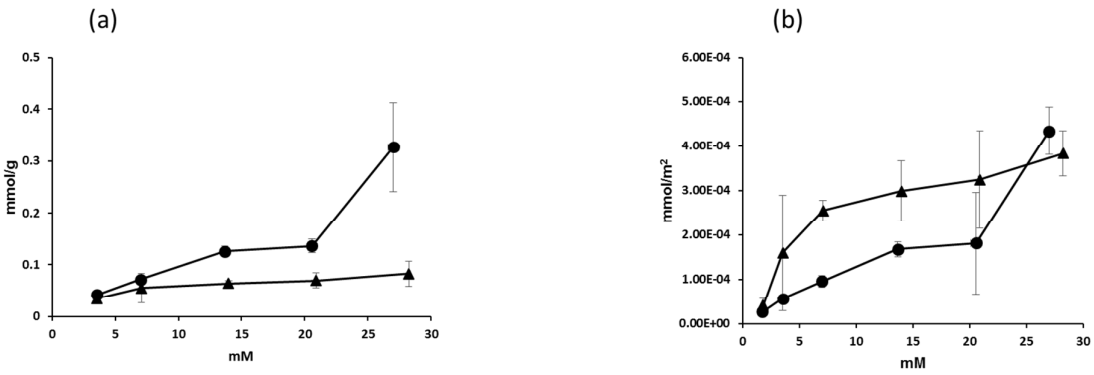


Figure 1. Adsorption isotherms for SZ adsorption ((a) mmol SZ /g silica and (b) mmol SZ/m^2 silica) onto SBA-15 (●) and Aerosil®200 (▲) at 24 h, 37°C in 0.1 M HCl (n=3, X and Y error bars indicate standard deviation)

Table 3. Isotherm parameters obtained by fitting sulphamethazine and sodium dodecyl sulphate adsorption data (mmol/m^2) onto SBA-15 and Aerosil®200 to Langmuir and Freundlich isotherms (SDS adsorption data only produced an acceptable fit with Freundlich isotherm). Measure of fit of data to model is indicated by the R^2 value.

SULPHAMETHAZINE			
Langmuir Isotherm	**N_t (mmol/m^2)**	**a (mM)**	**R^2**
SBA-15	0.04	0.0004	0.95
Aerosil®	0.15	0.0004	0.99
Freundlich isotherm	**m**	**K_o (mM)**	**R^2**
SBA-15	0.50	5.66	0.98
Aerosil®	0.70	8.09	0.91
SODIUM DODECYL SULPHATE			
Freundlich isotherm	**m**	**K_o (mM)**	**R^2**
SBA-15	0.53	10.70	0.98
Aerosil®	0.77	6.72	0.95

3.3.2. SDS Adsorption onto Silica in 0.1 M HCl Medium

The isotherm for SDS adsorption onto both silica substrates in 0.1M HCl at 37°C is presented in Figure 2. The quantity of surfactant adsorbed onto both silica materials was similar in magnitude to the quantity of drug adsorbed under the same experimental conditions (Figure 1 (b) *versus* Figure 2). For SBA-15 a correlation of $r = 0.83$ ($p < 0.04$) between surfactant and drug adsorption was determined while the correlation of adsorption on the non-porous surface was stronger at $r = 0.88$ ($p < 0.02$). The Freundlich adsorption model emerged as the best-fit model for SDS adsorption onto both substrates ($R^2 \geq 0.95$, Table 3). The Freundlich binding affinity for the surfactant with the mesoporous SBA-15 was stronger than that of the drug molecule. This is most likely a result of the surfactant's ability to reduce interfacial tension leading to improved pore wetting and access to additional binding sites in the porous network.

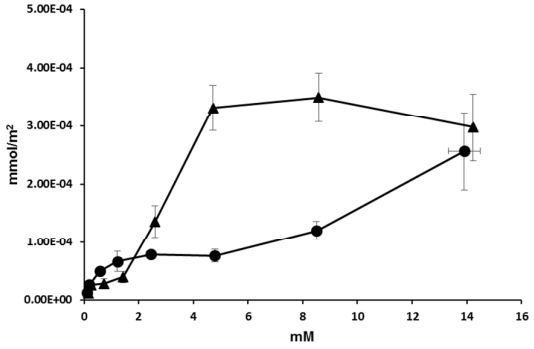


Figure 2. Adsorption isotherms for SDS adsorption (mmol SDS/m^2 silica) onto SBA-15 (●) and Aerosil (▲) at 24 h, 37°C in 0.1 M HCl (n=3, X and Y error bars indicate standard deviation)

3.3.3. Sulphamethazine Adsorption onto Silica in 0.1 M HCl/SDS Media

Adsorption isotherms for sulphamethazine adsorption (*mmol SZ/m^{2}silica*) onto SBA-15 and Aerosil® at 24 h in media with 0.1 M HCl (10 mM SDS) and 0.1 M HCl (50mM SDS) at 37^{o}C are displayed in Figure 3. There is significantly less drug adsorbed onto both silica materials in the presence of surfactant at both SDS concentrations investigated. Similar to drug adsorption in 0.1M HCl media without surfactant (Figure 1), the non-porous Aerosil® adsorbed a larger fraction of drug/m^2 than the mesoporous material. As this experiment involved a multi-component system where drug and surfactant are simultaneously adsorbing onto the silica surface, data was not fitted to the Freundlich and Langmuir adsorption models.

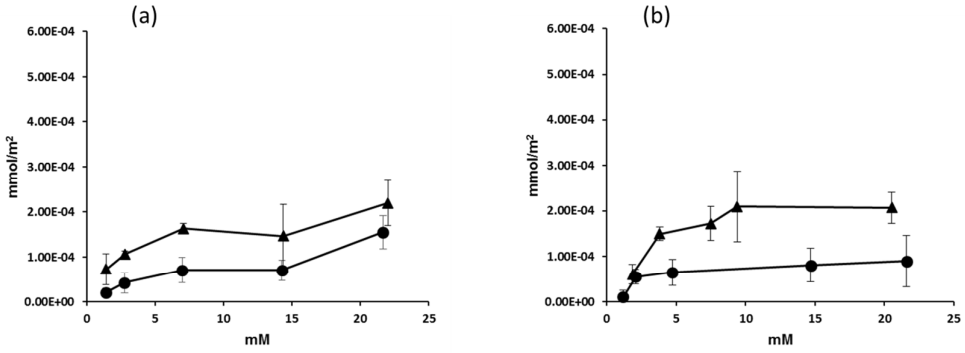


Figure 3. Adsorption isotherms for SZ adsorption (mmol SZ /m^2 silica) onto SBA-15 (●) and Aerosil (▲) at 24h, 37^{o}C in (a) 0.1M HCl (10mM SDS) and (b) 0.1M HCl (50mM SDS) (n=3, X and Y error bars indicate standard deviation)

3.4. Dissolution Studies

Dissolution experiments were conducted in the same three media as used for adsorption experiments. Experiments were conducted under sink conditions and the theoretical SZ concentration following 100% release was < 4% the SZ solubility in all cases (Table 4).

Table 4. Solubility and dissolution parameters for unprocessed SZ and SZ loaded silica formulations in the three dissolution media investigated (mean ± standard deviation is provided, n=3)

Dissolution Medium	Solubility	Dissolution	Dissolution (% Cumulative Release)				
	Solubility of Drug (mM)	% Saturated Solubility assuming 100% release	Sample	5 min	10min	15 min	24 h
0.1M HCl	30.00 ± 1.80	3.60	Unprocessed SZ	25.61±4.34	51.86±5.02	82.41±5.56	97.26 ± 1.80
			SZ loaded SBA-15	74.03±7.21	76.06±7.53	74.55±6.10	79.58±2.08
			SZ loaded Aerosil®	70.10±0.35	73.47±0.83	74.17±0.38	77.21±0.01
0.1M HCl 10mM SDS	30.28 ± 0.97	3.56	Unprocessed SZ	98.65±1.04	97.77±0.40	97.10±0.18	97.55±1.02
			SZ loaded SBA-15	90.20±0.65	90.27±1.06	90.32±0.86	92.94±1.25
			SZ loaded Aerosil®	89.13±4.75	90.84±4.09	89.08±4.41	86.15±5.23
0.1M HCl 50 mM SDS	38.80 ± 0.40	2.70	Unprocessed SZ	100.93±0.94	100.11±0.80	99.22±0.94	99.71±1.06
			SZ loaded SBA-15	86.97±3.15	91.95±3.03	94.97±6.33	98.02±4.44
			SZ loaded Aerosil®	91.84±5.26	92.84±5.27	92.53±4.74	90.40±4.12

3.4.1. Sulphamethazine/Silica Systems in 0.1 M HCl Medium

Loading SZ onto porous and non-porous silica carriers significantly enhanced the drug's dissolution rate in 0.1 M HCl buffer media compared to the unprocessed SZ (Figure 4 and Table 4). At the 5 min time point, SZ release from Aerosil®200 and SBA-15 was significantly higher than for the unprocessed drug. However, by 15 min, unprocessed SZ dissolution had significantly exceeded drug release from both silica systems. The amount of the free drug released remained higher for the unprocessed SZ than that of the drug/silica samples for the remainder of the experiment. At 24 h, incomplete drug release was observed for both silica systems; unprocessed SZ release was significantly greater than the extent of release from drug/silica samples (Table 4). Drug release from the porous and non-porous silica carriers was not significantly different at any of the dissolution time points.

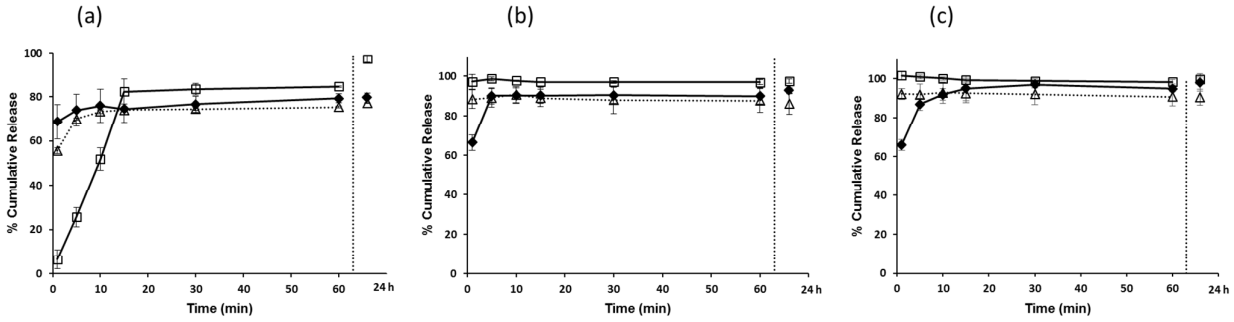


Figure 4. Dissolution profiles of SZ loaded SBA-15 (♦), Aerosil®200 (Δ) and unprocessed SZ (□) in (a) 0.1M HCl, (b) 0.1M HCl SDS 10mM and (c) 0.1M HCl SDS 50mM (n=3, Y error bars indicate standard deviation)

3.4.2. Sulphamethazine/Silica System in 0.1 M HCl/SDS Media

In vitro drug dissolution was investigated in the presence of surfactant (SDS) at the same concentrations investigated in the adsorption study (10 mM and 50 mM). The addition of the surfactant at low concentration (10 mM) significantly enhanced the rate and extent of drug release from both silica systems compared to dissolution in 0.1 M HCl media alone (Figure 4, Table 4). A further enhancement in the rate or extent of SZ release was not observed for the higher concentration of SDS (50 mM). Incomplete dissolution was observed for both porous and non-porous systems in the presence of 10 mM SDS (unprocessed drug dissolution reaches levels of 100% API release). Complete drug release was only observed for the drug/ SBA-15 samples in 0.1 M HCl containing 50mM SDS.

3.5. Porosity Analysis of Recovered SBA-15 Following Dissolution

Pore size distributions of unprocessed and recovered SBA-15 samples are displayed in Figure 5. Changes in silica porosity can indicate a change in the quantity and distribution of bound molecules on the silica surface. A decrease in pore diameter and pore volume is evidence of the presence of drug/surfactant molecules in the pores or blocking the pores [26]. SBA-15 samples recovered after dissolution in 0.1 M HCl displayed a reduction in mesopore volume but not mesopore width. This finding supports the hypothesis that a fraction of the drug molecules remaining is distributed on the silica surface after dissolution rather than blocking pore openings. Samples exposed to media containing surfactant displayed the greatest reduction in mesopore volume and demonstrated a significant reduction in mesopore size. This suggests that SDS molecules can adsorb onto the silica surface and have the potential to deposit in the silica mesopores and block them.

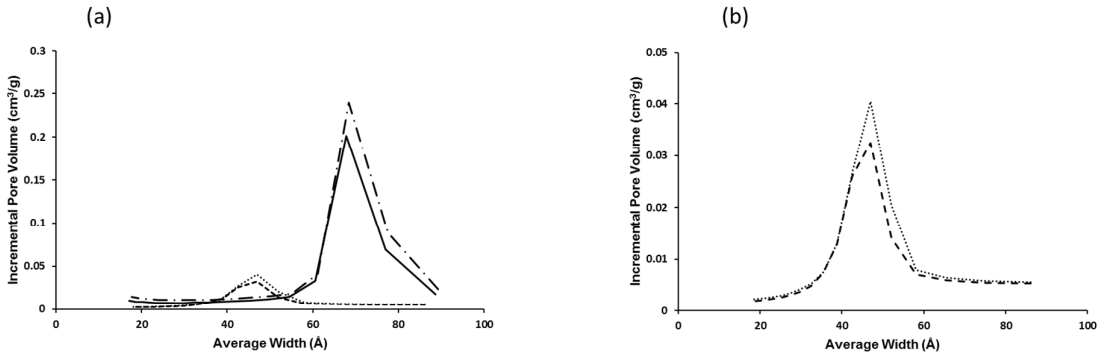


Figure 5. (a) Pore size distribution of unprocessed SBA-15 (dashed line with dot) and recovered SBA-15 samples after drug loading and dissolution in 0.1M HCl (black line), 0.1M HCl 10mM SDS (dotted line) and 0.1M HCl 50mM SDS (dashed line); 5(b) recovered SBA-15 samples after drug loading and dissolution in 0.1M HCl 10 mM SDS (dotted line) and in 0.1M HCl 50 mM SDS (dashed line)

3.6. Relating Dissolution Release Profiles to Adsorption Isotherms

The relationship between the quantity of SZ adsorbed on the silica surface at the end of the dissolution experiment and the estimated quantity of SZ adsorbed (calculated using the adsorption isotherm equations) was compared for the 0.1 M HCl media (Figure 6(a)). Figure 6(a) demonstrates that the quantity of drug that remains adsorbed to the mesoporous silica surface after dissolution is significantly higher than the predicted value. The amount retained per m^2 was considerably higher for the porous SBA-15 compared to non-porous Aerosil®. These results indicate that retention of drug molecules on the mesoporous silica surface was not simply due to an adsorption equilibrium between adsorbed drug and drug existing in solution in the dissolution media and that the porous architecture of silica influences the retention of drug on its surface.

In contrast to drug molecule adsorption behaviour, the quantity of SDS bound at the end of the dissolution experiment was not significantly different to the predicted values from the adsorption isotherms for the porous or non-porous systems (Figure 6 (b)).

The presence of SDS significantly reduces the amount of drug retained on the silica surface at the end the of the dissolution experiment (Figure 7). This is particularly evident for the mesoporous SBA-15. It is possible that the increased wettability of the media containing the surfactant provides enhanced access to drug binding sites, resulting in less drug retention. Increasing the concentration of SDS does not result in a significant further reduction in drug retention. While the presence of surfactant increases the extent of SZ dissolution, incomplete release was observed in dissolution experiments for both silica substrates (except SBA-15 loaded samples in 50mM SDS). This indicates that some drug molecules are so tightly bound to particular silica binding sites that they are, in essence, 'irreversibly bound' under the dissolution experimental conditions.

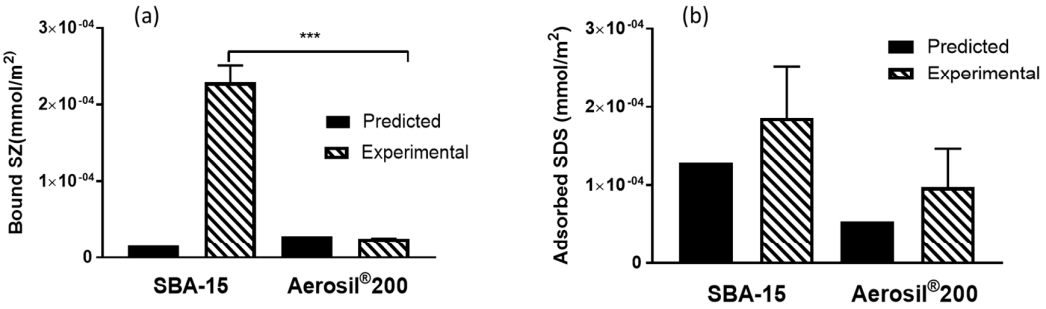


Figure 6. Comparison of (a) SZ and (b) SDS bound both predicted (from adsorption isotherm data) and experimentally determined after dissolution in 0.1M HCl (n=3, error bars indicate standard deviation, *** denotes $p < 0.001$)

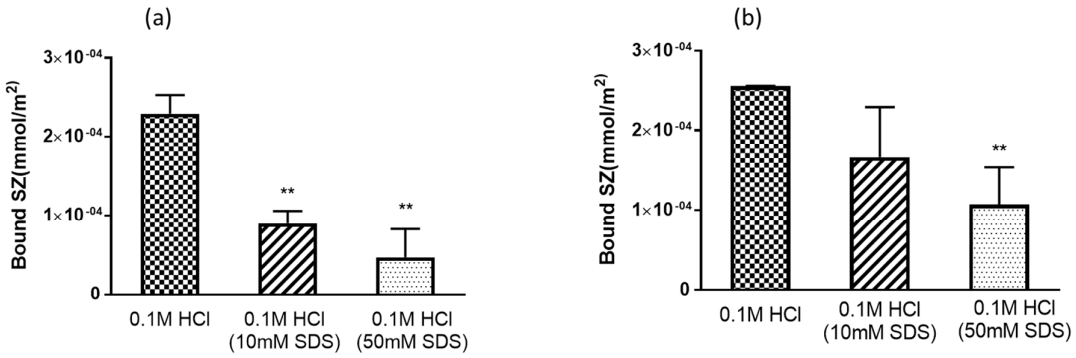


Figure 7. Comparison of the actual bound SZ fraction (*mmol/m^2 silica*) after dissolution in the three media investigated for (a) SBA-15 and (b) Aerosil® (n=3, error bars indicate standard deviation, ** denotes $p < 0.01$ of difference compared to amount bound in 0.1 M HCl)

5. DISCUSSION

In this study, two factors demonstrated a significant influence on drug release from silica systems. The first factor was the influence of drug and surfactant adsorption onto the silica surface. The second was the ability of the dissolution medium to wet the silica surface, particularly the porous network of the mesoporous SBA-15.

Drug adsorption onto the silica surface was noted for both silica materials across all three media investigated. The mesoporous material had a lower adsorbed drug fraction/m^2 compared to the non-porous Aerosil®. This indicates that SZ molecules cannot access the entirety of the mesoporous network. Mesoporous silica materials have a wide range of pore sizes (between 2 – 50 nm) [3]. It is possible that adsorbed drug molecules could block smaller pores preventing access to further drug binding sites located deeper in the porous architecture. Additionally, porous binding sites may be different in terms of the number of available sites/m^2 and/or binding affinity to those located on the surface, resulting in altered drug adsorption levels compared to non-porous materials. Further evidence for this hypothesis is observed in the porosity analysis which displays a reduced pore volume for the drug loaded samples after dissolution in 0.1 M HCl, indicating bound drug molecules remaining are occupying mesopores on the surface. This finding is interesting as it suggests accessible surface area rather than specific surface area of the SBA-15 is as an important parameter in drug loading and dissolution from these porous systems. Future studies should examine the influence of mesoporous materials with different porous architectures on drug adsorption and release from these formulations.

Adsorption isotherms for single component systems were fitted to the Langmuir and Freundlich linearized equations. These two models have also been used successfully in other studies investigating adsorption on silica substrates [16, 27, 28]. The Langmuir model describes a

homogeneous surface which contains only one type of binding site [29]. It emerged as the best-fit for drug binding onto the non-porous Aerosil®. In contrast, SZ adsorption onto mesoporous SBA-15 produced a Freundlich model best-fit correlation. The Freundlich model is an empirical model which describes a heterogeneous surface (a system which contains a range of binding sites with different binding affinities) and indicates that multi-layer adsorption of drug onto the porous SBA-15 surface exists [30]. This observation agreed with a previous literature report which demonstrated that the Freundlich isotherm proved the best-fit for the absorption of a range of pharmaceuticals onto SBA-15 [16]. The number of SZ binding sites on the surface (N_t) was lower for the mesoporous material. This is further evidence that the accessible surface area of the porous silica is an important parameter to consider for these formulations. The binding affinity (designated as a for the Langmuir isotherm and K_o for the Freundlich model) of drug to the surface is stronger for the non-porous Aerosil®. This is most likely a result of SBA-15's porous architecture as drug interactions with the surface could vary depending on the dimensions of the pores and silica surface chemistry.

The quantity of surfactant adsorbed onto both silica substrates was significantly similar in magnitude to the quantity of drug adsorbed under the same experimental conditions and concentration range (from adsorption isotherms, Figure 1(b) and Figure 2). This was determined by correlating drug and surfactant adsorption onto both silica surfaces (Section 3.3.2). Both molecules have a similar molecular mass (278.33g/mol for SZ and 288.372g/mol for SDS). SDS is an anionic surfactant and SZ has an aromatic amine functional group with a pK_a of 2.06 ± 0.30. The isoelectric point of the silica surface has been measured as pH 2 [31]. In 0.1M HCl, the results indicate that the more positively charged silica has a similar potential to attract both surfactant and drug molecules. Drug interaction with the silica surface is most likely a result of hydrogen bonding between the aromatic amine functional group and the silanol hydroxyl groups. Amine-silanol hydrogen bonding between drug molecules and the

silica surface has been reported previously in a study by Xue *et al* [17]. The interaction of the surfactant with the silica surface is not well understood. In this case, it is likely that the negatively charged head group interacts with the silica surface (which is slightly positively charged under these experimental conditions). It is possible that the SDS molecules adsorb in a multilayer hemi-micelle formation. This phenomenon has been described for cationic surfactant adsorption at the silica gel – water interface [32]. The nature of this surfactant – silica interaction requires further investigation.

The results of this study indicate that the retention of drug molecules on the mesoporous silica surface is not simply due to an equilibrium adsorption related to the concentration of drug in solution in the dissolution media. A certain fraction of the loaded drug molecules are bound very tightly or to sites which are inaccessible for the dissolution media. These findings reinforce the influence of the porous network in drug dissolution from these systems. While the quantity of loaded drug retained at the end of dissolution was greater than the predicted quantity, the amount of surfactant adsorbed was not significantly different when predicted and experimental values were compared. The surfactant was not loaded onto the silica material in the dissolution experiment. This observation indicates that the drug loading process utilised in this study (SC-CO_2 loading) is another factor to consider in determining drug adsorption/release behaviour. It has been reported in the literature that water molecules can adsorb onto silica by interacting with surface functional groups [33]. It is possible that during drug loading under SC-CO_2 conditions, water that was bound to the silica surface was removed thus activating potential binding sites which would otherwise be unavailable in the mesoporous material. This could increase the bound drug fraction remaining at the end of dissolution as drug molecules are potentially more difficult to remove from these binding sites.

While both concentrations of SDS investigated were determined to be above the surfactant CMC in 0.1M HCl (0.8mM), drug solubility was only marginally enhanced in the dissolution media containing 50mM SDS. This is most likely due to the extent of incorporation of SZ (an acidic drug) into surfactant micelles which is dependent on the pK_a (acid dissociation constant) of the drug and the ionic nature of the surfactant [34]. As the pK_a of the aromatic amine (2.06 ± 0.30) is only marginally above the pH, it is possible the drug is not fully protonated, reducing drug partitioning into anionic SDS micelles.

However, despite the marginal improvement in drug solubility, the addition of surfactant at both concentrations (10 mM and 50 mM) significantly enhanced the rate and extent of drug release from both porous and non-porous systems compared to dissolution in 0.1 M HCl alone. In this case, the improved wetting characteristics of the media in the presence of the surfactant is the most likely explanation for the improved dissolution profile. Surfactants decrease the solid/liquid surface tension [35] which could allow the dissolution media to access additional drug binding sites thus enhancing drug release. Superior release was observed for the non-porous Aerosil® at 1 min compared to SBA-15. This could be attributed to the time taken for the media to wet the pores. At 5 min, there is no significant difference in the extent of release between the two silica systems. This remains the case for the remainder of the experiment (24 h).

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6. CONCLUSIONS

This study demonstrates that drug adsorption plays a role in the release of drug molecules from drug/silica systems. Adsorption isotherms proved useful for understanding drug release for non-porous silica formulations. However, adsorption behaviour does not explain the high quantity of drug retained on mesoporous formulations. The addition of sodium dodecyl sulphate to the dissolution media was shown to have a significant impact on sulphamethazine dissolution from both porous and non-porous silica systems. The study findings highlight the importance of considering drug and dissolution media interaction with the silica substrate and accessibility of dissolution media to the silica porous architecture when optimising drug release from drug/silica systems.

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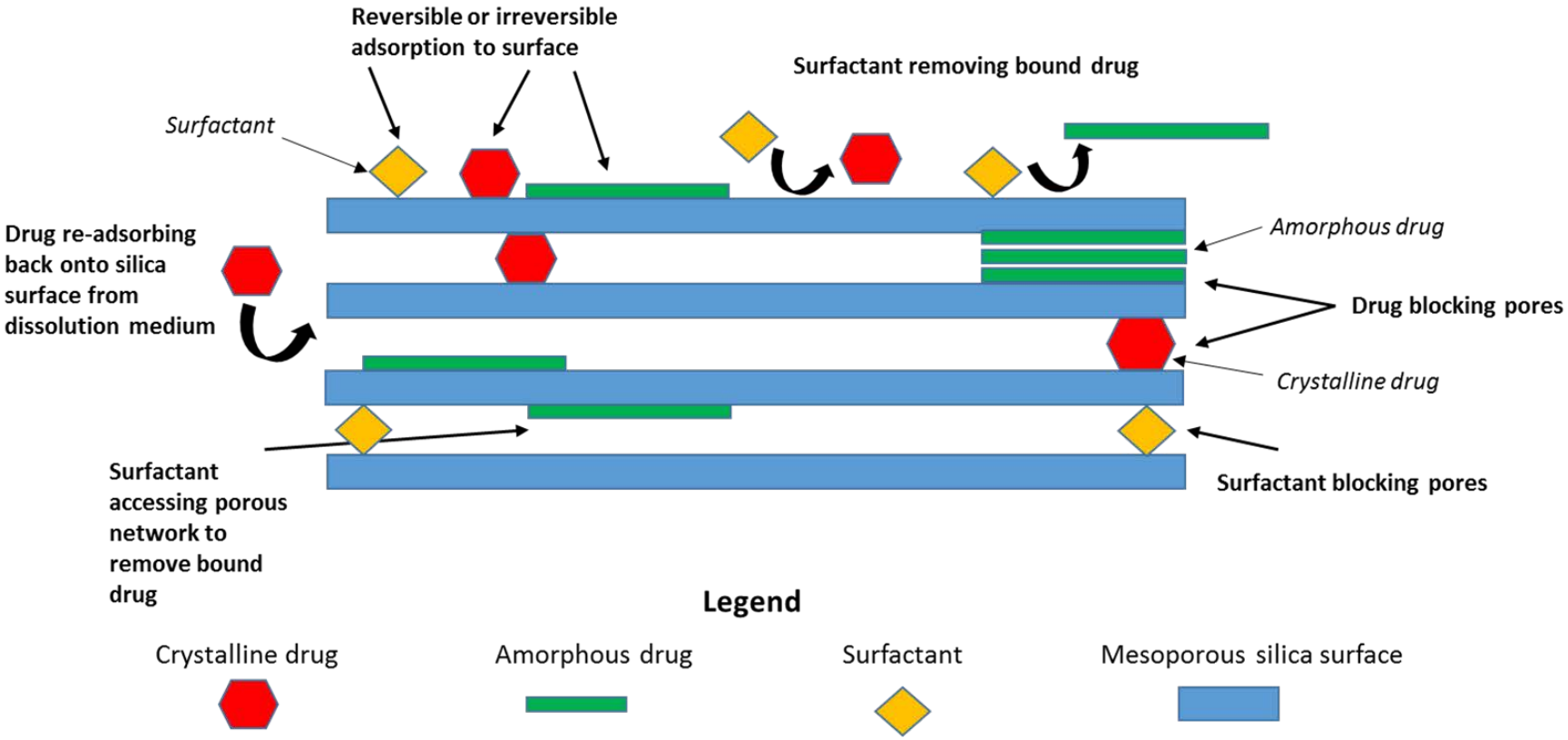
8. REFERENCES

1. Vallet-Regi, M.; Ramila, A.; Del Real, R.; Perez-Pariente, J. A new property of MCM-41: drug delivery system. *Chemistry of Materials* **2001,** *13*, (2), 308-311.
2. Mellaerts, R.; Jammaer, J. A. G.; Van Speybroeck, M.; Chen, H.; Van Humbeeck, J.; Augustijns, P.; Van den Mooter, G.; Martens, J. A. Physical state of poorly water soluble therapeutic molecules loaded into SBA-15 ordered mesoporous silica carriers: A case study with itraconazole and ibuprofen. *Langmuir* **2008,** *24*, (16), 8651-8659.
3. McCarthy, C. A.; Ahern, R. J.; Dontireddy, R.; Ryan, K. B.; Crean, A. M. Mesoporous silica formulation strategies for drug dissolution enhancement: a review. *Expert opinion on drug delivery* **2016,** *13*, (1), 93-108.
4. Kresge, C.; Leonowicz, M.; Roth, W.; Vartuli, J.; Beck, J. Ordered mesoporous molecular sieves synthesized by a liquid-crystal template mechanism. *nature* **1992,** *359*, (6397), 710-712.
5. Azaïs, T.; Tourné-Péteilh, C.; Aussenac, F.; Baccile, N.; Coelho, C.; Devoisselle, J.-M.; Babonneau, F. Solid-state NMR study of ibuprofen confined in MCM-41 material. *Chemistry of materials* **2006,** *18*, (26), 6382-6390.
6. Charnay, C.; Bégu, S.; Tourné-Péteilh, C.; Nicole, L.; Lerner, D. A.; Devoisselle, J. M. Inclusion of ibuprofen in mesoporous templated silica: drug loading and release property. *European Journal of Pharmaceutics and Biopharmaceutics* **2004,** *57*, (3), 533-540.
7. Shen, S. C.; Ng, W. K.; Chia, L.; Dong, Y. C.; Tan, R. B. Stabilized amorphous state of ibuprofen by co-spray drying with mesoporous SBA-15 to enhance dissolution properties. *Journal of pharmaceutical sciences* **2010,** *99*, (4), 1997-2007.
8. Ahern, R. J.; Hanrahan, J. P.; Tobin, J. M.; Ryan, K. B.; Crean, A. M. Comparison of fenofibrate–mesoporous silica drug-loading processes for enhanced drug delivery. *European Journal of Pharmaceutical Sciences* **2013,** *50*, (3–4), 400-409.
9. Limnell, T.; Santos, H. A.; Mäkilä, E.; Heikkilä, T.; Salonen, J.; Murzin, D. Y.; Kumar, N.; Laaksonen, T.; Peltonen, L.; Hirvonen, J. Drug delivery formulations of ordered and nonordered mesoporous silica: comparison of three drug loading methods. *Journal of pharmaceutical sciences* **2011,** *100*, (8), 3294-3306.
10. Waters, L. J.; Hussain, T.; Parkes, G.; Hanrahan, J. P.; Tobin, J. M. Inclusion of fenofibrate in a series of mesoporous silicas using microwave irradiation. *European Journal of Pharmaceutics and Biopharmaceutics* **2013,** *85*, (3), 936-941.
11. McCarthy, C. A.; Faisal, W.; O'Shea, J. P.; Murphy, C.; Ahern, R. J.; Ryan, K. B.; Griffin, B. T.; Crean, A. M. In vitro dissolution models for the prediction of in vivo performance of an oral mesoporous silica formulation. *Journal of Controlled Release* **2017,** *250*, 86-95.
12. Van Speybroeck, M.; Mellaerts, R.; Mols, R.; Thi, T. D.; Martens, J. A.; Van Humbeeck, J.; Annaert, P.; Van den Mooter, G.; Augustijns, P. Enhanced absorption of the poorly soluble drug fenofibrate by tuning its release rate from ordered mesoporous silica. *European Journal of Pharmaceutical Sciences* **2010,** *41*, (5), 623-630.
13. Bukara, K.; Schueller, L.; Rosier, J.; Daems, T.; Verheyden, L.; Eelen, S.; Martens, J. A.; Van den Mooter, G.; Bugarski, B.; Kiekens, F. In Vivo Performance of Fenofibrate Formulated With Ordered Mesoporous Silica Versus 2-Marketed Formulations: A Comparative Bioavailability Study in Beagle Dogs. *Journal of Pharmaceutical Sciences* **2016,** *105*, (8), 2381-2385.
14. Van Speybroeck, M.; Barillaro, V.; Thi, T. D.; Mellaerts, R.; Martens, J.; Van Humbeeck, J.; Vermant, J.; Annaert, P.; Van den Mooter, G.; Augustijns, P. Ordered mesoporous silica material SBA-15: A broad-spectrum formulation platform for poorly soluble drugs. *Journal of pharmaceutical sciences* **2009,** *98*, (8), 2648-2658.
15. Ahern, R. J.; Crean, A. M.; Ryan, K. B. The influence of supercritical carbon dioxide (SC-CO< sub> 2</sub>) processing conditions on drug loading and physicochemical properties. *International journal of pharmaceutics* **2012,** *439*, (1), 92-99.

16. Bui, T. X.; Choi, H. Adsorptive removal of selected pharmaceuticals by mesoporous silica SBA-15. *Journal of Hazardous Materials* **2009,** *168*, (2), 602-608.
17. Xue, J. M.; Shi, M. PLGA/mesoporous silica hybrid structure for controlled drug release. *Journal of Controlled Release* **2004,** *98*, (2), 209-217.
18. Leuner, C.; Dressman, J. Improving drug solubility for oral delivery using solid dispersions. *European Journal of Pharmaceutics and Biopharmaceutics* **2000,** *50*, (1), 47-60.
19. U.S. Food and Drug Administration Dissolution Methods. http://www.accessdata.fda.gov/scripts/cder/dissolution/ (02/03/2017),
20. Swearingen, R. A.; Chen, X.; Petersen, J. S.; Riley, K. S.; Wang, D.; Zhorov, E. Determination of the binding parameter constants of Renagel® capsules and tablets utilizing the Langmuir approximation at various pH by ion chromatography. *Journal of pharmaceutical and biomedical analysis* **2002,** *29*, (1), 195-201.
21. García-Calzón, J. A.; Díaz-García, M. E. Characterization of binding sites in molecularly imprinted polymers. *Sensors and Actuators B: Chemical* **2007,** *123*, (2), 1180-1194.
22. Ding, S.; Chen, J.; Jiang, H.; He, J.; Shi, W.; Zhao, W.; Shen, J. Application of Quantum Dot−Antibody Conjugates for Detection of Sulfamethazine Residue in Chicken Muscle Tissue. *Journal of Agricultural and Food Chemistry* **2006,** *54*, (17), 6139-6142.
23. Im, S. H.; Ryoo, J. J. Characterization of sodium laureth sulfate by reversed-phase liquid chromatography with evaporative light scattering detection and 1H nuclear magnetic resonance spectroscopy. *Journal of Chromatography A* **2009,** *1216*, (12), 2339-2344.
24. Barrett, E. P.; Joyner, L. G.; Halenda, P. P. The determination of pore volume and area distributions in porous substances. I. Computations from nitrogen isotherms. *J. Am. chem. soc* **1951,** *73*, (1), 373-380.
25. Rangel-Yagui, C. O.; Pessoa Jr, A.; Tavares, L. C. Micellar solubilization of drugs. *J Pharm Pharm Sci* **2005,** *8*, (2), 147-163.
26. Izquierdo-Barba, I.; Martinez, Á.; Doadrio, A. L.; Pérez-Pariente, J.; Vallet-Regí, M. Release evaluation of drugs from ordered three-dimensional silica structures. *European Journal of Pharmaceutical Sciences* **2005,** *26*, (5), 365-373.
27. Andersson, J.; Rosenholm, J.; Areva, S.; Lindén, M. Influences of material characteristics on ibuprofen drug loading and release profiles from ordered micro-and mesoporous silica matrices. *Chemistry of Materials* **2004,** *16*, (21), 4160-4167.
28. Smirnova, I.; Mamic, J.; Arlt, W. Adsorption of Drugs on Silica Aerogels. *Langmuir* **2003,** *19*, (20), 8521-8525.
29. Umpleby, R. J.; Baxter, S. C.; Chen, Y.; Shah, R. N.; Shimizu, K. D. Characterization of molecularly imprinted polymers with the Langmuir− Freundlich isotherm. *Analytical chemistry* **2001,** *73*, (19), 4584-4591.
30. Umpleby Ii, R. J.; Baxter, S. C.; Bode, M.; Berch Jr, J. K.; Shah, R. N.; Shimizu, K. D. Application of the Freundlich adsorption isotherm in the characterization of molecularly imprinted polymers. *Analytica Chimica Acta* **2001,** *435*, (1), 35-42.
31. Kosmulski, M. Positive Electrokinetic Charge of Silica in the Presence of Chlorides. *Journal of Colloid and Interface Science* **1998,** *208*, (2), 543-545.
32. Gao, Y.; Du, J.; Gu, T. Hemimicelle formation of cationic surfactants at the silica gel–water interface. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases* **1987,** *83*, (8), 2671-2679.
33. Vigil, G.; Xu, Z.; Steinberg, S.; Israelachvili, J. Interactions of Silica Surfaces. *Journal of Colloid and Interface Science* **1994,** *165*, (2), 367-385.
34. Park, S.-H.; Choi, H.-K. The effects of surfactants on the dissolution profiles of poorly water-soluble acidic drugs. *International Journal of Pharmaceutics* **2006,** *321*, (1–2), 35-41.
35. Rosen, M. J.; Kunjappu, J. T., *Surfactants and interfacial phenomena*. John Wiley & Sons: 2012.


Reversible or irreversible adsorption to surface
Surfactant removing bound drug
Surfactant
Drug re-adsorbing back onto silica surface from dissolution medium
Amorphous drug
Drug blocking pores
Crystalline drug
Surfactant accessing porous network to remove bound drug
Surfactant blocking pores
Legend
Crystalline drug
Amorphous drug
Surfactant
Mesoporous silica surface