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Bioinspired Silica Offers a Novel, Green, and Biocompatible Alternative to Traditional Drug Delivery Systems

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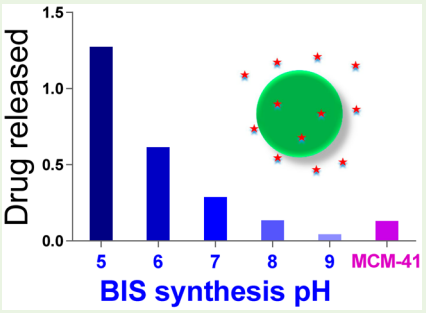
S *Supporting Information*

ABSTRACT: Development of drug delivery systems (DDS) is essential in many cases to remedy the limitations of free drug molecules. Silica has been of great interest as a DDS due to being more robust and versatile than other types of DDS (e.g., liposomes). Using ibuprofen as a model drug, we investigated bioinspired silica (BIS) as a new DDS and compared it to mesoporous silica (MS); the latter has received much attention for drug delivery applications. BIS is synthesized under benign conditions without the use of hazardous chemicals, which enables controllable in situ loading of drugs by carefully designing the DDS formulation conditions. Here, we systematically studied these conditions (e.g., chemistry, concentration, and pH) to understand BIS as a DDS and further achieve high loading and release of ibuprofen. Drug loading into BIS could be enhanced (up to 70%) by increasing the concentration of the bioinspired additive. Increasing the silicate concentration increased the release to 50%. Finally, acidic synthesis conditions could raise loading efficiency to 62% while also increasing the total mass of drug released. By identifying ideal formulation conditions for BIS, we produced a DDS that was able to release fivefold more drug per weight of silica when compared with MCM-41. Biocompatibility of BIS was also investigated, and it was found that, although ∼20% of BIS was able to pass through the gut wall into the bloodstream, it was nonhemolytic (∼2% hemolysis at 500 μg mL^{-1}) when compared to MS (10% hemolysis at the same concentration). Overall, for DDS, it is clear that BIS has several advantages over MS (ease of synthesis, controllability, and lack of hazardous chemicals) as well as being less toxic, making BIS a real potentially viable green alternative to DDS.



KEYWORDS: *nanomaterials, nanomedicines, pharmaceuticals, cytotoxicity, biomedical devices*

1. INTRODUCTION

Drug molecules currently on the market, while effective, can have a whole range of limitations which reduce the efficacy of the drug. Some limitations include poor solubility, in vivo degradation, and short systemic circulation times.[1] Due to these factors, to achieve efficacy, drugs may require higher doses, which can result in higher toxicity.[1] One method of improving drug efficacy is by developing drug delivery systems (DDS).[2] Aside from the obvious potential medicinal benefits of DDS, there are also large economic benefits to be gained as new DDS take significantly less time and investment to develop than a new drug molecule (3−4 years and approximately $20−50 million for DDS[1] vs 10−12 years and $500 million for a new drug[3]).

Many materials have been investigated for the use as DDS, e.g., liposomes, polymeric nanoparticles (e.g., dendrimers), and "hard" nanoparticles, mainly consisting of metals (e.g., gold), metal oxides (e.g., iron oxide, titanium oxide, and silica) or carbon.[4−6] However, relatively few DDS are currently on the market.[7] The main limitations for any DDS becoming a clinical product are the long regulatory journey coupled with issues with biocompatibility, efficacy, and the manufacturing processes. Briefly, a DDS must first be proven to work and be safe in vitro and then in vivo, manufacture should be straightfor-

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ward, and it should provide significant benefits over risks before it can gain support from patents and financial backing. Next, human clinical trials are carried out and, if these are passed, then the product will go on to become commercialized.[7] This long, multistep process can create obstacles for new DDS and result in the failure of many of them. Due to the high failure rate of DDS, there is huge potential for new developments in this field.

Here, we focus on silica as a DDS because there has been increasing interest in the use of silica nanoparticles for the purpose of drug delivery since 2001, when Vallet-Regi et al. described the effective loading and release of ibuprofen from a type of mesoporous silica nanoparticle (MCM-41).[8] The successful use of a silica DDS over other systems (e.g., liposomes) has been attributed to its thermal and chemical stability as well as versatility compared to those of conventional drug delivery systems.[9−12] Further, silica offers a versatile platform for functionalization with biomolecules to tailor drug release as well as targeted delivery. One of the most common methods of controlling drug release is through functionalizing silica to create stimuli-responsive DDS. This opens up a wide range of external stimuli which can be used to manipulate these materials, ranging from magnetism, ultrasound, and light to the more conventional temperature and pH.[13−16] Functionalization has also shown promise in targeted drug delivery. For example, an interesting avenue is using silica functionalized with cell-penetrating peptides for targeted delivery directly into cytoplasm.[17]

Silica can be functionalized with various chemical groups, making it compatible with a range of drugs. Examples of various drugs investigated with silica range from anti-inflammatories such as ibuprofen or aspirin, antibiotics such as gentamicin and erythromycin, and antimalarials and anticancer drugs such as doxorubicin and campothecin.[8−10,18−27] While a gold-coated silica product (Auroshell[28]) is in the first stage of development as an anticancer agent, there are currently no silica-based drug delivery systems on the market, despite the fact that MS showed some promise as an effective DDS nearly 15 years ago. This delay is due to several limitations, including long and laborious synthesis (synthesis of MCM-41 can take between 10 and 146 h[29−32]) and use of harsh chemicals, toxic surfactants, hazardous precursors, and harsh conditions (extreme temperatures and pH[31]). These imply that drug loading can occur only postsynthesis, which adds another step (and extra time) to the synthesis of this type of DDS. Therefore, a greener, economical, scalable, and safer method of synthesizing silica with potential for in situ drug loading is highly favorable.

Biomineralization of silica is observed in several species of aquatic unicellular organisms such as diatoms (a class of algae)[33] as well as in more complex organisms such as some sponge species and even in some plants.[34−36] It was found that specific proteins and biomolecules were involved in the condensation of biosilica such as silicatein and silaffin.[34−36] By understanding the chemistry and role of these biomolecules, we developed analogues of these biomolecules ("additives", typically amines) which have been shown to rapidly condense silica under benign conditions.[37,38] As such, this has enabled discovery of bioinspired silica (BIS) which can be controllably synthesized at room temperature, at a neutral pH, in water, and within 5 min.[39] This also opens the possibility of in situ drug loading, thus allowing a one step, green DDS formulation.[40] Further, amine-ibuprofen interactions have been reported to be favorable for drug delivery,[41−43] which provides another potential benefit of BIS over MS: a possible additional function of the amine additives.

As yet, only five papers have been published on the use of BIS synthesis for drug delivery applications (including one from our group[40]), suggesting a vast potential for future research. Li et al. utilized a so-called "biomimetic" synthesis route; however, this method retained all of the issues of synthesizing MCM-41 (i.e., long synthesis time, high temperatures, and requirement for calcination).[44] Begum et al. made use of surfactants to create porosity; thus, their system still requires an energy intensive calcination step as well as postsynthesis drug loading.[45] Sano et al. designed a drug molecule which had the dual function of pharmacological activity and silica condensation ability (not all drug molecules will have this dual ability), meaning that the system was limited to only a small set of drug molecules.[46] Lechner et al. linked their cargo molecule to a silica condensing peptide; however, they were not able to fully control drug release. Conjugation of a peptide with a drug has many other issues such as loss of drug activity, use of hazardous chemicals, and also an extra synthesis step.[47] Preliminary work from our group reported the green synthesis of silica with in situ drug loading of calcein (a hydrophilic drug-like molecule).[40] This synthesis required no calcination, and the amine additive was separate from the drug molecule. BIS did not show any significant toxic effects to either fibroblasts or human monocytes in the resting state even at high silica concentrations. However, mesoporous silica particles showed substantially reduced cell viability even at low concentrations. For example, the silica concentration required to reduce cell viability to 50% (IC_{50}) was 5−10 times more for BIS than for MCM-41. Further, BIS did not induce secretion of inflammatory cytokines at the concentrations proposed for use in DDS.[40] From these results, it is evident that, despite the use of amine additives, BIS is safe and does not cause concerning cytotoxicity.

In the present study, we aimed to further extend BIS to a pharmaceutically active drug molecule (ibuprofen) and create a DDS formulation which, through carefully investigating and understanding the formulation chemistry, would have the ability to control the loading and release of pharmaceutically active drugs. Ibuprofen was chosen because it is a commonly used model drug for DDS development due to its small molecular size (1.0 × 0.6 nm^2),[8] stability,[48] ease of detection (UV absorbance at ∼220 nm), and available literature on ibuprofen-silica systems for comparison. The main aim of this research is to primarily understand in situ drug loading into the BIS system. Specifically, we plan to determine predictive rules and investigate the effects of the amine additive, drug interactions, and silica chemistry on DDS performance (drug loading and release profiles). Further, to make BIS a viable DDS, it should exhibit loading and release profiles for ibuprofen similar to or improved over those of the competitor MCM-41-based DDS.

2. MATERIAL AND METHODS

2.1. Chemical Reagents. All reagents were purchased from Sigma unless otherwise stated. Reagents included: acetronitrile (HPLC Plus, ≥ 99.9%), ammonia (NH_3, anhydrous, ≥ 99.98%), anhydrous sodium sulphite (97%), ammonium molybdate·$4H_2O$, calcium chloride hexahydrate (USP testing specifications), concentrated hydrochloric acid, diethylenetriamine (DETA) (99%), dinitrophenol (≥98.0%) (DNP), Dulbecco's phosphate buffered saline (D-PBS), formic acid (≥95%), glucose (≥99.5%), heparin, hexadecyltrimethylammonium bromide (CTAB), hydrochloric acid solution 1 M (HCl, Fisher),

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ibuprofen (≥98%), Immu-mount, magnesium sulfate heptahydrate, oxalic acid·2H_2O (≥99.5%), pentaethylenehexamine (PEHA) (technical grade), potassium chloride (≥99.0%), PBS (tablets pH 7.4), poly(allylamine hydrochloride) average M_w ~17500 (PAH), poly(fluorescein isothiocyanate allyamine hydrochloride) (poly(allylamine hydrochloride):fluorescein isothiocyanate 50:1), potassium phosphate monobasic, sodium chloride (≥99.5%), sodium metasilicate pentahydrate (technical) (Fisher), sulfuric acid (98%), tetraehylenepentamine (TEPA) (Acros Organics), tetraethoxysilane (TEOS) (99.999% trace metal basis), and Triton X-100 (laboratory grade).

2.2. In Situ Drug Loading into BIS and Drug Release. To a solution of sodium metasilicate in deionized water was added a solution of amine additive (in water) followed by an ibuprofen solution (in 70% ethanol). Then, a known volume of 1 M HCl (the volume of HCl required varied depending on the amine additive used) was added to reduce the pH of the solution to the desired pH (7, unless otherwise stated). The concentrations of the reactants in the final solution were 30 mM sodium metasilicate, 1 mg mL^{-1} PAH, and 1 mg mL^{-1} ibuprofen: this ratio was termed 1:1:1. For a 50 mL batch of 1:1:1, 0.3182 g of sodium silicate, 0.05 mL of PAH, and 0.05 g of ibuprofen were used. When BIS was synthesized with other amines (DETA, TEPA, and PEHA), a molar ratio of 1:1 [Si]:[N] was used. This equates to 0.05155 g of DETA, 0.05678 g of TEPA, and 0.05809 g of PEHA for a 50 mL batch. Once acid was added, silica precipitated within seconds, and the solution was left for 5 min before being centrifuged at 8000 rpm for 15 min to stop the reaction. The supernatant was stored at 4 °C to determine the drug loading efficiency (percent of drug loaded into the silica) and drug content (percent weight of drug in the silica-drug complex) via the method described in Section 2.4. The silica pellet was resuspended, washed in deionized water, centrifuged two more times (no detectable drug was observed in these supernatants), and finally dried at 45 °C for at least 5 h.

Once dried, 10 mg of the silica was suspended in 1.4 mL of PBS (pH 7.4) and incubated at 37 °C to measure drug release. At each time point (1, 3, 5, 7, and 24 h time points), samples were centrifuged at 8000 rpm for 15 min, and 1 mL of the supernatant was used for high-performance liquid chromatography (HPLC) analysis and replaced with fresh PBS to satisfy the perfect sink conditions for determination of the diffusion parameters. Release is expressed as the percent of the loaded drug which has been released from 10 mg of silica. Each sample was prepared in triplicate, and release profiles were measured from each sample in triplicate.

2.3. Synthesis of MCM-41 and Postsynthesis Drug Loading. MCM-41 was synthesized by first dissolving CTAB in 300 mL of 25% ammonia at 35 °C. While being stirred, 20 mL of TEOS was slowly added. This solution was then stirred for 3 h and aged for 24 h at room temperature in a closed container to allow silica to form. The product was then vacuum filtered and washed with 1 L of distilled water and finally dried overnight at 85 °C. To remove the surfactant (CTAB), MCM-41 was calcinated at 500 °C for 5 h. This was based on previously published methods.[12]

To load the drug, 10 mg of MCM-41 was immersed in a 1 mg mL^{-1} solution of ibuprofen (in 70% ethanol) at 37 °C overnight. Samples were centrifuged at 8000 rpm for 15 min, and the supernatant was removed (and supernatant drug concentration was measured to determine loading efficiency) and replaced with fresh PBS for a release experiment. At each time point, samples were centrifuged at 8000 rpm for 15 min, and 1 mL of the supernatant was taken for HPLC analysis and replaced with fresh PBS.

2.4. Drug Detection via HPLC. Drug loading and release were determined via an HPLC analysis method. A DIONEX system was used with an autosampler (GINA50), pump (P580), and variable wavelength detector (UVD170S) along with an ACE 5 C-18 column (150 × 4.6 nm with 5 μm particle size) at room temperature. An isocratic reverse phase HPLC method was used with a 30 μL injection volume and a mobile phase of acetonitrile:0.1% formic acid (70:30) at a flow rate of 1 mL min^{-1}. Ibuprofen retention time was approximately 4.7 min and was detected at a wavelength of 220 nm (λ_{max} wavelength of ibuprofen). Data were collected using Chromeleon V6.80 software, and peaks were integrated to determine drug concentration. Data were fitted with a single exponential (eq 1) where Y_0 is the final release (%), A is a constant, and R_0 is the slope. When A and R_0 were multiplied, the maximum rate of release (% release per hour) was deduced.

$$y = Y_0 + Ae^{R_0 X} \quad (1)$$

2.5. Material Characterization. Silica samples were characterized using nitrogen adsorption in a micromeritics ASAP 2420 Accelerated Surface Area and Porosimetry system. Samples were first weighed and degassed in optimum pressure and temperature conditions (120 °C). They were then held at the boiling point of nitrogen and evacuated, allowing nitrogen gas to enter the sample tubes while the pressure was monitored. Analyses of the data included BET (Brunauer–Emmett–Teller[49]) theory, used to characterize the surface areas of the silica particles, and the BJH (Barrett–Joyner–Halenda[50]) theory, which allowed for the characterization of the silica pore size distributions.

Silica samples were imaged by scanning electron microscopy (SEM) using a Hitachi SU6600 field electron SEM. Samples were mounted on sample holders using sticky carbon tape and then gold splutter coated under vacuum to prevent charging of the sample. Micrographs were taken using a 20 kV potential difference and a working distance of 8.7 mm.

2.6. Measuring Movement of Silica across the Gut Wall. Rats (200–250 g, male, Sprague–Dawley) were anesthetized via intraperitoneal injection with pentobarbitone (60 mg/kg) and sacrificed for the experiment. The small intestine was removed and washed with 37 °C Krebs solution (made from distilled H_2O, 16.09% (w/v) NaCl, 1.1% (w/v) KCl, 0.22 M KH_2PO_4, 2.74% (w/v) $MgSO_4·7H_2O$, and 0.12 M $CaCl_2·6H_2O$). Intestines were then inverted and bathed in Krebs solution while ensuring the 37 °C temperature was kept constant. Small sections of gut (~5–6 cm) were cut and tied closed at one end with thread and filled with 1 mL of fresh Krebs solution, and then the open end was also tied closed.

To verify the health of the sections of gut, a control experiment was set up which measured the passage of glucose across the gut wall. Sections of gut were either immersed in 6 mL of a 1 mM glucose solution or in a 1 mM DNP (dinitrophenol) solution (to inhibit the active transport of glucose[51]) for 15 min at 37 °C before a glucose solution (to make a final concentration of 1 mM) was added. Sections of gut were then incubated at 37 °C for an hour before being cut open and their contents removed. Glucose concentrations were measured using a glucose (gluc-pap) assay kit purchased from Randox.

To measure the passage of silica through the gut wall, fluorescent silica was prepared using the same method as in Section 2.2 except that PAH-FITC was used as the amine additive, thus creating fluorescently labeled silica. Fluorescence was measured on a RF-530IPC fluorometer at an excitation wavelength of 495 nm and an emission wavelength of 515 nm. Tubes of inverted rat gut sections were incubated in a 1 mg/mL silica solution (in Krebs) or a 1 mg mL^{-1} silica solution and 1 mM DNP for an hour at 37 °C. Gut sections were then cut open, their contents removed, and their fluorescence measured; the sections were then fixed in a formalin solution (neutral buffered 10%) for 30 min followed by two PBS (pH 7.4) washes. The inside and outside surfaces of the gut sections were then imaged using a Carl Zeiss Axio Imager Z1 with 10×/0.30 lens. Sections of gut were mounted either by stretching the gut and pinning the edges or compressing gut sections under Immu-mount and coverslips.

2.7. Hemolytic Activity of Silica. To measure the hemolytic activity of silica, rats (Sprague–Dawley) were bled, and the blood was stabilized with heparin (100 μL of 1000 units ml^{-1}). Four milliliters of heparin stabilized blood was diluted with 9 mL of Dulbecco's PBS and centrifuged at 2250g for 5 min. The supernatant was carefully removed, and the blood was washed five times with D-PBS. After the last wash, the red blood cells (RBC) were diluted with 40 mL of D-PBS. Diluted RBC (0.2 mL) were then added to 0.8 mL of silica suspension at the desired concentration to make a final silica suspension. Positive and negative controls were set up by adding 0.2 mL of RBC to either 0.8 mL D-PBS or 0.8 mL of 0.2% Triton X-100. All samples were prepared in triplicate and briefly vortexed before

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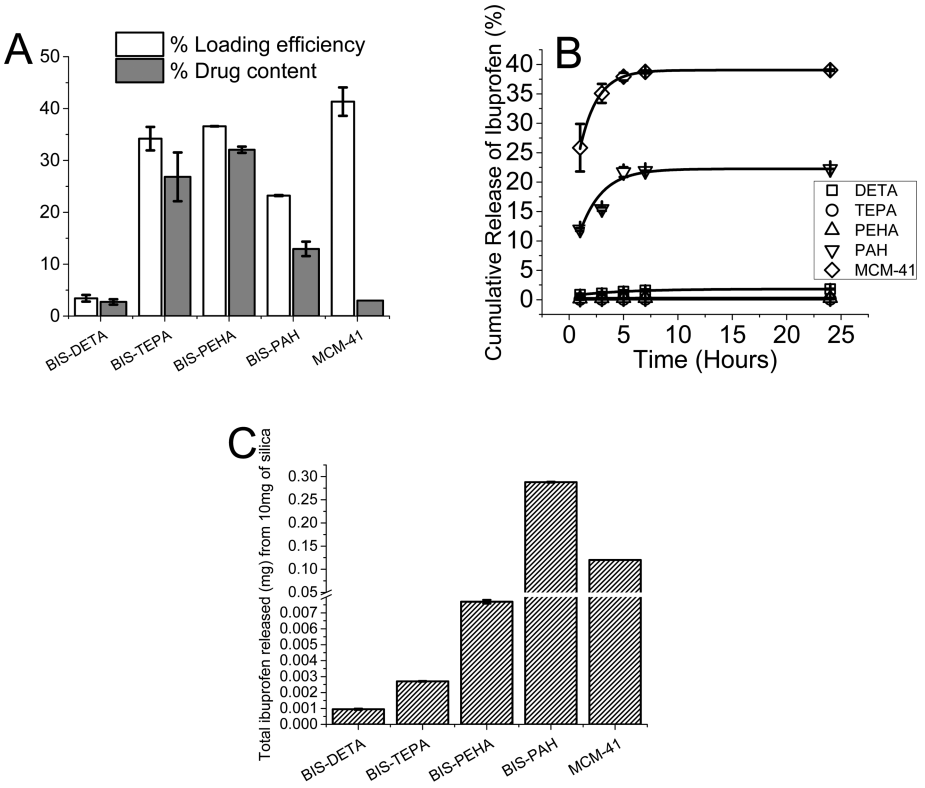

D	Surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
BIS-DETA	16	0.095	*
BIS-TEPA	33	0.095	*
BIS-PEHA	36	0.131	*
BIS-PAH	129	0.91	23
MCM-41	983	0.74	2

Figure 1. BIS synthesized with different amines. (A) Percent loading efficiency and percent drug content (w/w) of ibuprofen loaded into four different BIS and MCM-41 systems. (B) Percent release of loaded ibuprofen from four different BIS and MCM-1 systems. (C) Total mass of ibuprofen (mg) released from 10 mg of silica sample. (D) Surface area, pore volume, and pore size figures for four different BIS and MCM-41 systems (an asterisk represents that specific pore sizes are not applicable due to broad pore size distributions). For A, B, and C, $n = 3$; error bars represent one standard deviation. For D, $n = 1$.

being left static at room temperature for 4 h. Samples were then vortexed again and centrifuged at 10000*g* for 2 min. Supernatant (10 μL) was used to test the absorbance of hemoglobin using an anthos2020 plate reader at 577 nm with a reference wavelength of 655 nm. Hemolysis was calculated as % hemolysis = [(sample absorbance − negative control)/(positive control − negative control)] × 100.[52]

3. RESULTS AND DISCUSSION

In order for BIS to be developed as an effective DDS, one must understand the synthesis chemistry and the mechanisms that dictate the loading and release of drug molecules from the system. There has been little information published on the loading mechanics of the BIS system and it has been speculated, but not proven, that embedded amine, originally employed to facilitate silica condensation, also helps to functionalize the silica.[40] If this is the case, then the BIS DDS can be synthesized, functionalized, and drug-loaded all in one step, which is a vast improvement on the long multistep process involved in MS. All of these possibilities were investigated herein.

3.1. Effect of the Amine Additive on the Loading and Release of Ibuprofen. The effect of the choice of amine additive for the synthesis of BIS upon its ability to load and release calcein (a nonpharmaceutically active but "drug-like" molecule) has previously been reported.[40] As these effects are drug specific, we investigated them for an active drug molecule (ibuprofen) in the BIS system and compared earlier results for calcein with those for ibuprofen.

To screen the most suitable systems, four additives were investigated: three small amines and one polyamine. These were chosen based on their silica precipitation performance and previous investigations into BIS.[37,38,40,53] We measured the loading efficiency (amount of drug loaded on DDS when compared to the concentration used for loading), drug content in the DDS (amount of drug loaded per weight of DDS), and total amount of drug released (mg drug/10 mg DDS). DETA, a small amine, was immediately excluded for use as it had a

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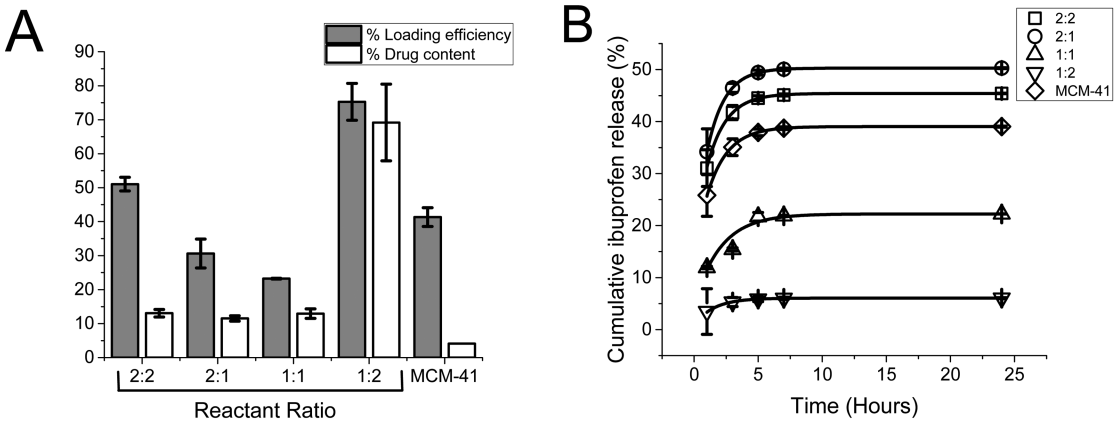

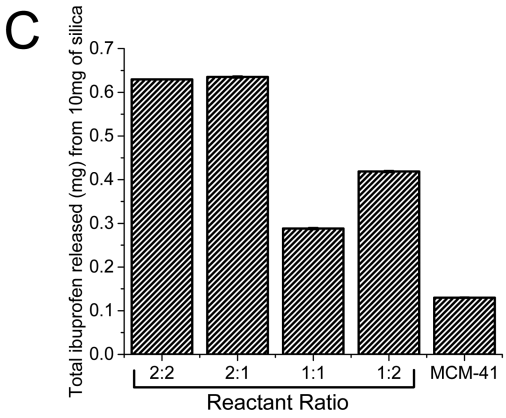

D	Surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
2:2	157	0.73	15.9
2:1	180	0.531	11.3
1:1	129	0.91	23
1:2	76	0.69	28.94
MCM-41	983	0.74	2

Figure 2. Effect of reactant concentrations on the loading and release profiles of ibuprofen. (A) Percent loading efficiency and percent drug content (w/w) of ibuprofen loaded into BIS synthesized with different reactant ratios and MCM-41. (B) Percent release of loaded ibuprofen from four different BIS and MCM-41 systems. (C) Total mass of ibuprofen (mg) released from 10 mg of silica sample. (D) Surface area, pore volume, and pore size figures for BIS synthesized with different reactant concentrations and MCM-41. For A–C, n = 3; error bars represent one standard deviation. For D, n = 1.

loading efficiency of only <5% (Figure 1A). The other amines used were PEHA, TEPA, and PAH, and they exhibited loading efficiencies of 20–30%, while MCM-41 showed ~40% loading efficiency (Figure 1A). These differences between BIS and MCM-41 are likely due to the different methods by which the drug was loaded into these two types of silica. For BIS, ibuprofen was loaded in situ, and the drug would have been entrapped within the silica particles followed by some surface physisorption. With MCM-41, only postsynthesis loading was possible, and drug loading was entirely reliant on physisorption (hence surface area and porosity is important in this system).

Focusing on drug release from these DDS, despite having loading efficiencies similar to those of BIS-PAH, BIS-TEPA and BIS-PEHA released <2% of the loaded drug, and as such, these amines must also be discarded (Figures 1B and C). Approximately 22% of loaded ibuprofen was released from BIS-PAH compared to the 39% released from MCM-41 (Figure 1B and Table S1). The release data appeared to fit well using a single exponential equation with >0.9 R^2 values in all cases (Table S1). The fitting showed that BIS-DETA, BIS-TEPA, and BIS-PEHA all had very low release rates (Figures 1B and C). However, the rates of release (Table S1) from MCM-41 and BIS-PAH were similar (15 and 17% per hour, respectively).

The drug loading efficiency on MCM-41 was found to be 41%, while the loading efficiency for BIS-PAH was 23% (Figure 1A). Despite this, MCM-41 released around half the amount of drug when compared to BIS-PAH (0.12 mg compared to 0.28 mg for 10 mg DDS, respectively). This implies that for a dose of 1 mg of ibuprofen, a patient would have to take ~83 mg of MCM-41 compared to only ~54 mg of BIS-PAH. High doses of MCM-41 silica can result in serious toxicity issues, unlike with BIS,[40,52] which highlights a key benefit of using BIS.

The differences in release profiles between BIS synthesized with the different amines are likely to be due to the porosity and morphology characteristics of the synthesized silica (Figures 1D and S1B). Adsorption of drugs is a function of pore size, pore volume, and surface area; particle size does not have any impact on release.[54,55] In the case of MCM-41 (a mesoporous silica), it is generally accepted that porosity is a major factor in controlling the release of drugs, and so further investigation was needed to determine whether this was the case for BIS.[43,56,57] BIS-DETA, TEPA, and PEHA all have a very small pore volumes (~0.1 cm^3/g) and low surface areas (~20–40 m^2/g) (Figure 1D). The pore volume and surface area for BIS-PAH (0.74 cm^3/g and 129 m^2/g, respectively) were higher than those of silica synthesized with the other three amines. This suggests that silica particles synthesized with any of the small amines were dense when compared to BIS-PAH, which explains the higher release from BIS-PAH within the BIS series. These observations explain why the BIS-DETA, TEPA, and PEHA samples exhibit poor drug loading/release when compared with BIS-PAH. Interestingly, MCM-41 has a much larger surface area (989 m^2/g) than any of the BIS samples but

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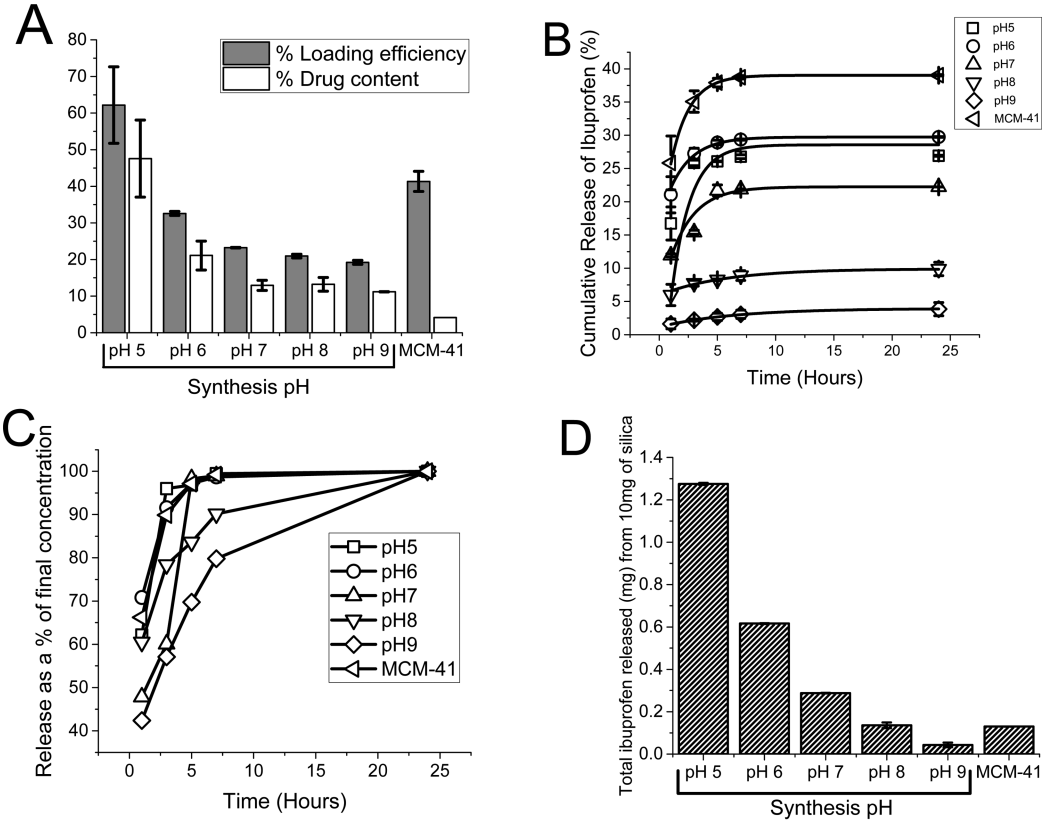

E	Surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
5	142	0.60	21
6	149	0.67	21
7	129	0.91	23
8	161	0.84	25
9	140	0.68	25

Figure 3. Effect of reaction pH on the loading and release profiles of ibuprofen. (A) Percent loading efficiency and percent drug content (w/w) of ibuprofen loaded into BIS synthesized at different pH and MCM-41. (B) Percent release of loaded ibuprofen from silica. (C) Release of loaded ibuprofen expressed as a percent of final concentration released from BIS synthesized at a range of pH values. (D) Total mass of ibuprofen (mg) released from 10 mg of silica sample. (E) Surface area, pore volume, and pore size figures for BIS synthesized at different pH value and MCM-41 samples. For A, B, and D, $n = 3$; error bars represent one standard deviation. For C and E, $n = 1$.

demonstrated loading efficiency comparable to that of BIS-PAH. SEM revealed that BIS-PAH particles were fairly uniform in shape and size, exhibiting a range between 72 ± 17 and 78 ± 18 nm (Figures S2A and B) without and with the drug, respectively, thus suggesting that the presence of the drug did not affect the particle sizes significantly. On the other hand, MCM-41 samples used herein were not only very large in comparison (3340 ± 1013 nm, Figures S1A and B) but also nonuniform with large variations in size and shape. Further, it is interesting to note that, despite the difference in particle size between MCM-41 and BIS-PAH, the amounts of drug released were often comparable. At this point in time, a direct comparison between these two DDS is not possible simply based on SEM results because of their distinctly different drug loading mechanisms, and further analysis in future is necessary.

Along with porosity altering the release of ibuprofen, it has been reported that the amine-ibuprofen interaction is important in loading.[40,42,58] Because BIS-TEPA and BIS-PEHA showed over 30% drug loading efficiency, it is possible that the amine additives facilitate ibuprofen loading through favorable amine-drug interactions as reported elsewhere[41−43,58] but that they also form nonporous silica by fully encapsulating ibuprofen within the dense silica particles, thus resulting in very low release. PAH, however, allows ibuprofen loading through favorable interactions with amine groups, and release occurs through the silica pores. These observations are consistent with the literature, where it has been reported that these small amines lead to the formation of dense and nonporous silica, while PAH forms porous silica.[37,38]

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3.2. Altering Reactant Concentrations To Understand the Silica-Drug System. The main aim here is to understand the DDS and investigate how controllable it is with ibuprofen so that this knowledge can be implemented for other drugs. As such, our next step was to study the effects of reaction chemistry on DDS performance. There has been some evidence that altering reactant concentrations can alter the loading and release profiles of calcein from BIS synthesized with PAH;[40] however, the reasons behind this effect were not fully investigated. Therefore, a systematic approach by varying synthesis conditions and evaluating their effects on drug loading and release has been taken while keeping the starting concentration of ibuprofen in the reaction mixture constant (1 mg mL^{-1}).

Figures 2A and Table S2 show that for MCM-41 (as reported in the section above), the loading efficiency was ∼40% and the drug content was ∼3 wt %. The loading efficiency and drug content for the 1:1 BIS-PAH sample (30 mM solution of sodium metasilicate and a 1 mg mL^{-1} solution of PAH) were ∼22% and 13 wt %, respectively. When the concentrations of silicate and PAH were doubled (2:2), there was a doubling of ibuprofen loading efficiency (Figure 2A). This was attributed simply to more silica being formed (Table S2) because the drug content did not change (Figure 2A). When only the silicate concentration was increased and the PAH concentration was kept at 1 mg mL^{-1} (2:1), there was a slight increase in ibuprofen loading efficiency (Figure 2A), but drug content remained unchanged, which was attributed simply due to an increased silica yield (Table S2). Producing more silica means that more ibuprofen was loaded (and so less was wasted by being left in the reaction mixture). Interestingly, when a synthesis ratio of 1:2 (increasing PAH concentration but maintaining silicate concentration) was investigated, drug loading efficiency increased 3-fold to 75% (Figure 2A). This loading efficiency (which was significantly higher than that found for MCM-41 (∼40%)) was produced from a significantly lower silica yield (Table S2). The drug content also increased substantially from ∼10% for 1:1 to ∼70% for 1:2. This is likely due to a drug-amine interaction, suggesting that the amine can have a dual function of facilitating silica condensation as well as acting as a functionalization agent to facilitate drug loading (see Section 3.3 for further discussion). These loading studies highlight that the synthetic conditions can readily modulate the loading efficiency of BIS and even reach loadings that are significantly higher than what is achievable with MCM-1.

Finally, the release of ibuprofen from these samples was investigated, and it was found that the overall release of ibuprofen from different silica varied. BIS-PAH (1:1) released 22% of the loaded ibuprofen, and 2:2 and 2:1 both achieved higher releases (45 and 50%, respectively), which were greater than the 39% released from MCM-41 (Figures 2B and C). It is possible that release from 2:2 and 2:1 was higher than that from 1:1 due to faster silica condensation because the silica precursor concentration used was doubled.[59] This resulted in lower pore volumes and smaller pores (Figures 2D and S3B), leading to less drug being entrapped within the silica and remaining mainly as surface bound, making release easier. In contrast, a 1:2 ratio released only 6% of loaded ibuprofen (Figure 2B) despite a very high loading efficiency and larger pore size (Figures 2D and S3B).

When the release profiles were considered (Figure S3A), all but the 2:1 samples exhibited burst release, where the majority of drug was released over the first 5 h and very little release was observed after this point (Table S2). This suggests that the ibuprofen that is able to escape is mainly surface bound and any ibuprofen embedded within the silica particles is trapped and unable to be released. This idea is supported by Figure S3A where all the BIS release profiles were similar to the release profile of MCM-41, which only had surface bound ibuprofen loaded. However, the 1:2 system had a much lower maximum release rate than the other systems (Table S2) as well as low total release (Figure 2B). Table S2 also shows that the mass of ibuprofen released from all the BIS systems were higher than from MCM-41, some BIS samples releasing 5x more drug per weight of silica than MCM-41. This is important since if more mg of drug is released then less silica will need to be administered to a patient.

3.3. Understanding Additive-Drug Interactions To Control DDS Formulation. Ibuprofen contains a carboxylic acid group, which is expected to interact with amines. Several studies have exploited these favorable amine-ibuprofen interactions by postsynthetically functionalizing MS.[41−43,58] In addition, from the results presented above, there was an indication that the PAH-ibuprofen interactions are important for drug loading and release. Therefore, we investigated whether drug loading and release could be controlled by tuning PAH-ibuprofen interactions by varying the synthesis pH (and in turn the protonation). In this study, silica was usually formed at pH 7 as silica formation is the quickest at neutral pH for this synthesis method.[40,59] BIS will not readily form outside the pH ranges of 5−9; hence, we focused on exploring drug loading under this pH range and monitored the effect of formulation pH on the drug release (Figure 3).

When silica was condensed at pH ≥ 7, the loading efficiency was not altered (remaining at ∼20%, Figure 3A). When the synthesis pH was more acidic, on the other hand, ibuprofen loading efficiency could be enhanced up to three times (to 60%) at pH 5. A similar picture was observed for the drug content (wt %) shown in Figure 3A. The release for samples formulated at pH ≤ 7 was similar (Figure 3C and Table S3), whereas DDS formulated at pH ≥ 7 had greatly diminished release. It should be noted that all release experiments were carried out in PBS at pH 7.2. Interestingly, despite the higher drug loading at pH 5, there was no corresponding higher release observed when compared with DDS formulated at pH 7 (Figure 3B). Despite this, the total ibuprofen (mg) released per weight of silica for the pH 5 sample was 10 times higher than that for the MCM-41 sample (Figure 3D)

When release was plotted as a fraction of total release over time, two different release profiles became apparent (Figure 3C). BIS-PAH synthesized at pH ≤ 7 exhibited a burst release profile similar to those observed for BIS samples reported above (also evident from high release rates, Table S3), where the majority of ibuprofen was released from the silica in the first 5 h, and very little was released after this. This burst release profile was similar to that seen for MCM-41, suggesting that the main mechanism for release in these systems was release from the surface. However, silica synthesized at pH > 7 appeared to have a slow and sustained release profile, which was also reflected in slow release rates (Table S3). Release did not plateau for 24 h, and ibuprofen maintained a slow release over the course of the experiment. This slow release suggested that the loaded ibuprofen was embedded within the silica rather than bound to the surface, making release more prolonged. While the total amount of ibuprofen released from these

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samples under the 24 h observation window was low, this system does show some promise as a prolonged release system.

It is clear from the results presented that the DDS formulation pH controlled the loading and release of ibuprofen. This could be caused by differences in porosity, morphology, and/or additive-drug interaction. SEM results suggested that pH did not have a significant effect on the morphology or particle sizes of DDS (Figures S2A and B). When surface area and pore volume were measured for BIS-PAH DDS formulated at different pH conditions, there were no significant differences observed (Figures 3E and S4). The differences in ibuprofen loading in these systems can then likely be attributed to ionization of the three components present (silica, amine additive, and drug) in the reaction mixture as well as the silica formation pathways. A scheme showing how the proportions of ionized reactants vary as the reactant pH is altered can be seen in Figure 4 and Table S4. The results here suggest that the

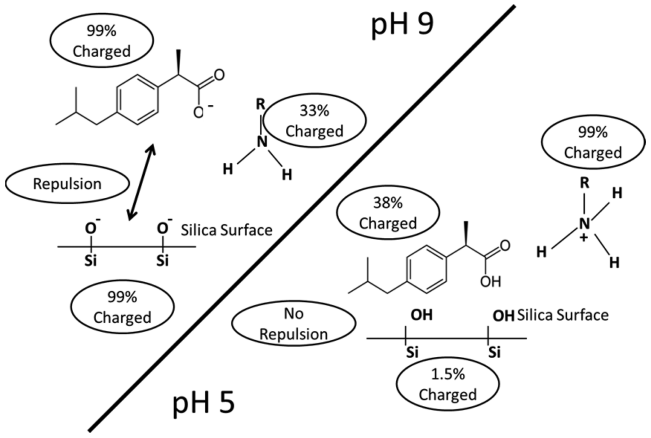

Figure 4. Scheme to illustrate the differences in charge of silica, amine, and ibuprofen during synthesis at pH values ranging from 9 to 5.

negative charge on silica can have an inhibitory effect on loading efficiency. Both the silica surface and ibuprofen are negatively charged at pH ≥ 7 (Table S4), and therefore silica and the drug will repel one another, thus explaining low loading efficiencies at pH ≥ 7 (only ∼20% of ibuprofen was loaded under these conditions, Figure 3A and Table S3). With DDS formulations prepared under acidic conditions, and particularly at pH 5, the silica and ibuprofen are both significantly less charged, thus allowing ibuprofen to be more efficiently loaded (30−60% of ibuprofen was loaded under acidic conditions, Figure 3A and Table S3).

It is clear that pH has a drastic effect on the loading efficiency of ibuprofen into BIS, with more acidic conditions resulting in increased loading. There is also strong evidence of an amine-drug interaction playing a major role in the ability of BIS to load the drug. This interaction, when too strong, can also inhibit drug release.

3.4. Biocompatibility of BIS. Due to the ease and noninvasive nature of administration, oral delivery of drugs is the most preferred route for patients.[60] Silica is an ideal material for oral drug delivery due to its stability under the conditions found in the gastrointestinal tract, especially the low pH found in the stomach (pH 1−3), and therefore it is able to protect the loaded drug molecules from the changes in pH as well as degradative enzymes and bile salts.[61,62] While amine functionalization is beneficial for drug loading and controlling release, exposure of amine-functionalized MS to cells has been reported to result in a higher cytotoxicity,[40] higher level of plasma membrane damage, and higher hemolytic activity.[52] BIS was reported to be either noncytotoxic, toxic only at extremely high concentrations, or when internalized into activated macrophages.[40] To further improve our understanding of BIS, it is important to uncover the fate of orally administered silica.

A simple and effective experiment was set up using sections of rat gut and measuring the movement of fluorescently tagged BIS-PAH (FITC-BIS-PAH) across the gut wall over an hour. FITC-BIS-PAH was synthesized using FITC-tagged PAH so that its movement through the gut wall could be measured. We observed that ∼22% of silica moved across the gut wall during the hour-long incubation (Figure 5). This movement was

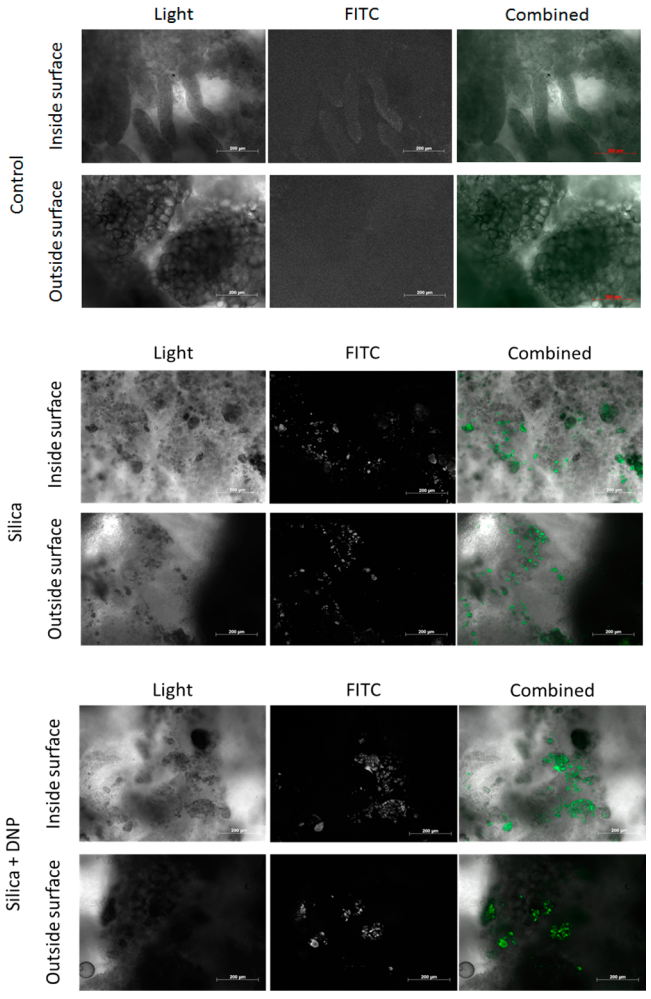

Figure 5. Light and FITC microscopy images of the inside and outside surfaces of rat gut incubated with no silica (control), fluorescent silica, or fluorescent silica and DNP. Images were taken using a Carl Zeiss Axio Imager Z1 with a 10×/0.30 lens.

through passive diffusion because it was not affected by the addition of an inhibitor of active transport (DNP). To further observe the movement of silica particles through the gut wall, fluorescence microscopy images of the inner and outer surfaces of the rat gut were taken (Figure 5). It is clear that when no silica is present, there are no defined points of fluorescence, but in the gut sections exposed to silica and silica with DNP, defined points of silica are observed. Silica was clearly localized on both sides of the gut wall, confirming its movement. Due to

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the ability of BIS-PAH to pass through the gut wall, it became important to investigate its biocompatibility with other cell types, particularly RBC.

The effect of BIS on RBC was determined through hemolytic activity of BIS when exposed to RBC. Figure 6 shows that BIS-PAH had very low hemolytic activity, lysing only 2% of RBC at the highest concentration used (500 μg/mL) and only 0.6% at the concentration which passed through the gut wall (~250 μg/mL). MCM-41 exhibited a higher hemolytic activity, rising to 10% at 500 μg/mL. The reasons behind this difference were initially unclear but may be related to the size of the particles. It has been reported that silica particle size affects hemolysis.[63] BIS-PAH particles were spherical (78 ± 18 nm in diameter, Figures S2A and B) and significantly smaller than the irregular MCM-41 particles used (3340 ± 1013 nm in diameter), which could partly explain the difference in hemolytic activity between BIS and MCM-41. SEM data also show that, although BIS primary particles were <100 nm, they form micrometer-sized agglomerates and rapidly precipitate (hence DLS was not possible or useful). It is thus expected that BIS particles are as toxic as MCM-41 simply based on their sizes, but this was not observed. Although further work is required on BIS to fully understand their biocompatibility, our present and previous results show that BIS is more biocompatible than MS.

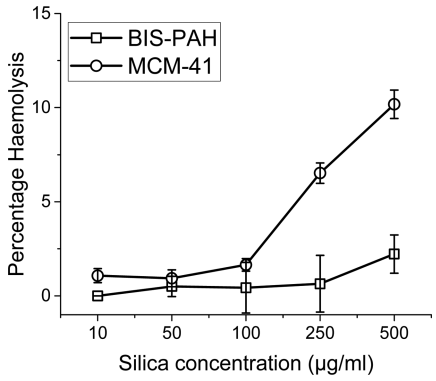

Figure 6. Percentage hemolysis induced by varying concentrations of BIS-PAH and MCM-41 after 1 h incubation with red blood cells. n = 3, error bars represent one standard deviation.

4. CONCLUSIONS

Our primary aim was to develop an in situ drug loading and release system using BIS. The BIS system can be controlled using many factors such as the choice of amine additive, pH of synthesis, kinetics of synthesis, and eventual location of the drug within the silica (Figure 7). Our results identified that the ideal formulation is BIS-PAH synthesized with a reactant ratio of 2:2. Formulation under an acidic pH was found to be suitable for designing DDS for faster targeted release, while basic pH was preferred for sustained release (Figure 7). Although a small portion of BIS-PAH was able to pass through the gut wall into the bloodstream, due to its low hemolytic activity, that does not appear to be an issue, in contrast to MCM-41. Ultimately, BIS appears to have several advantages over MCM-41 (such as one step formulation, simple controllability, and lack of hazardous chemicals), and it was found that BIS has drug loading and release profiles similar to or improved over those of MCM-41 in addition to superior biocompatibility. These benefits give BIS real potential as a viable DDS to be further investigated. We believe that the understanding of the DDS formulation using BIS that has emerged from this work can enable the discovery and development of a wide variety of DDS.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomaterials.6b00224.

Data for loading and release profiles, mathematical fitting of release data, release presented as percent of final

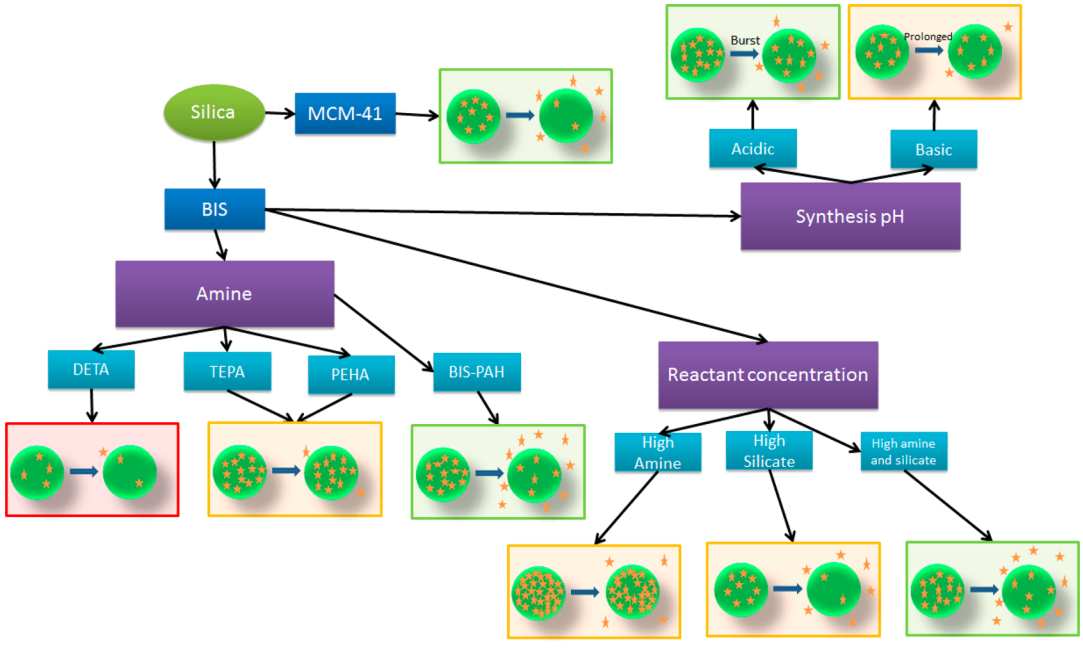

Figure 7. Schematic summary of results presented in this paper.

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concentration and pore size distributions for BIS synthesized with different amines, reactant concentrations, and pH values, SEM images, particle size measurements, and percent ionization data (PDF)

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The authors declare no competing financial interest.

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ABBREVIATIONS

BIS, bioinspired silica; BIS-PAH, bioinspired silica synthesized with poly(allylamine hydrochloride); CTAB, hexadecyltrimethylammonium bromide; DDS, drug delivery system; DETA, diethylenetriamine; DLS, dynamic light scattering; DNP, dinitrophenol; D-PBS, Dulbecco's phosphate buffered saline; HPLC, high-performance liquid chromatography; MCM-41, Mobil Composition of Matter No. 41; MS, mesoporous silica; PAH, poly(allylamine hydrochloride); PAH-FITC, poly(fluorescein isothiocyanate allyamine hydrochloride); PBS, phosphate buffered saline; PEHA, pentaethylenehexamine; RBC, red blood cell; SBA-15, Santa Barbara Amorphous type material 15; SEM, scanning electron microscope; TEOS, tetraethoxysilane; TEPA, tetraehylenepentamine; UV, ultraviolet

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