The Open Access Journal of Science and Technology
vol. 5 (2017), Article ID 101190, 18 pages
doi:10.11131/2017/101190

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Research Article

Adsorption and Elution of Nucleic Acids: Mesoporous Materials and Methods

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Abstract. Physical protection of nucleic acids from the environment for improved stability through encapsulation or adsorption has been reported using various materials, including liposomes, metal particles, mesoporous silica nanoparticles, and polymers. In an extension of that approach, our previous study demonstrated the potential of mesoporous silica nanoparticles with and without covalently attached stabilizing reagents, such as sugar and bovine serum albumin, for improving the stability of RNA. The goal of the current study is to evaluate the potential of silicate sorbents bearing chemical functionalities for stabilization of nucleic acid targets. Materials offering charged groups, metal chelating sites, and π-bonding sites are considered. Adsorption and elution of RNA, DNA, and single stranded DNA (ssDNA) are evaluated as is subsequent elution of the bound target. A sorbent functionalized with primary amine groups showed promising results for RNA and ssDNA stabilization. The impact of the sorbents on long term viability the targets is also evaluated. Storage of adsorbed targets at room temperature and 37°C over a period of 200 d indicates the potential for stabilization of RNA and ssDNA using several of the functionalities. None of the sorbents improved the stability of DNA either under room temperature or 37°C storage.

Keywords: organosilicate; DNA; RNA; stability; capacity

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Dates
Received 30 November 2015
Accepted 19 January 2017

1. Introduction

Successful advancements in molecular diagnostics related to a wide range of fields, including medical, biological, environmental, forensics, and food safety, drive the need for preservation of nucleic acid integrity during sample collection, transportation, processing, and storage. The most common method for maintaining nucleic acid integrity is freezing at low temperature (−20°C or −80°C). This approach is not practical for routine specimen processing, storage, or shipping related to austere field conditions. Furthermore, the costs associated with maintaining large sets of samples under the necessary conditions over long periods of time can be prohibitive [1–3]. To address these issues, several technologies have been developed for the stabilization and storage of nucleic acids at room temperature. These technologies are primarily based on three principles. The first is anhydrobiosis, the dehydration process used by some organisms to

survive extreme conditions [4, 5]. Anhydrobiosis based methods are commonly used for DNA and include spray drying, spray-freeze-drying, air drying, and lyophilization with or without additives (i.e. trehalose) for preservation [6–12]. One study also indicated that anhydrobiosis worked well for RNA preservation [13]. While in the dry state, matrix components form a thermo-stable barrier around the DNA protecting the sample from damage and degradation. DNA can be recovered by rehydration as the matrix will completely dissolve [1, 14]. The second approach to stabilization is to use chemicals or proteins to bind nucleic acids; this changes the characteristics of interactions with the nucleic acids to provide stability. Several chemicals and compounds have been reported to preserve nucleic acids at room temperature from periods of weeks to months. DNA-binding protein from starved cells (Dps) and poly(A) binding protein (Pab1p) were reported to stabilize DNA and mRNA, respectively [3, 15–30]. Commercial products, such as RNAlater and Trizol (ThermoFisher Scientific, Waltham, MA, USA), are based on this approach and have been documented to stabilize nucleic acids at room temperature for long periods of time [16, 26, 31–34].

Physically protecting nucleic acids from the environment, through encapsulation or adsorption onto a solid support, is the third of the stabilization principles and has emerged for the delivery of gene therapeutics. Materials, such as liposomes, metal particles, mesoporous silica nanoparticles, polymers, potato starch, silk fibron and surfactants, have been developed with these applications in mind [35–47]. In a previous study, we demonstrated the potential of RNA adsorption onto mesoporous silica nanoparticles (MSNs) with and without additional stabilizing reagents. The MSNs provided enhanced stability for extended periods allowing the adsorbed RNA to be eluted using simple buffers and employed directly for downstream molecular diagnostic assays. While promising, the RNA recovery rates from these MSNs were not ideal (less than 25%) [48]. The goal of the ongoing effort is to control interactions with the nucleic acids that result in degradation. Some of these interactions are restricted through adsorption or encapsulation; for example, the access of enzymes and microorganisms as well as the mobility of the nucleic acids. Other features of interest for the sorbents are altering solvent interactions, providing reducing sites and chelating groups, and inhibiting nuclease activity. While the previous study focused on common sugars and bovine serum albumin as stabilizing agents, the study presented here uses chemical functionalities incorporated into the sorbents to provide the potential for addressing other aspects of nucleic acid degradation. Binding and elution of RNA, DNA, and single stranded DNA (ssDNA) are evaluated. Elution methods are compared and the impact of the sorbents on long term sample viability is assessed.

2. Experimental Section

1,2-bis(trimethoxysilyl)ethane 96% (BTE), mesitylene (1,3,5-trimethylbenzene or TMB), 2-(N-morpholino)ethanesulfonic acid sodium salt (MES), cetyltrimethylammonium bromide (CTAB), PAMAM-25% C12 dendrimer (ethylenediamine core, generation 4.0 solution 10 wt. % in methanol), zinc acetate, copper chloride, chitosan (low molecular weight), *N*(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), tetraethyl orthosilicate (TEOS), tetramethyl orthosilicate 98% (TMOS), Pluronic®P123 (P123), nitric acid 70%, and hydrochloric acid 37% were obtained from Sigma-Aldrich (St. Louis, MO, USA). Bis(trimethoxysilylethyl) benzene (DEB, mixture of 1,4 and 1,3 isomers), 3-

aminopropyltrimethoxysilane (APS), phenyltrimethoxysilane (PTS), N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (EDA), 3-isocyanatopropyltriethoxysilane (ICS), *N*-trimethoxysilylpropyl-*N,N,N*-trimethylammonium chloride (TSPMC, 50% in methanol), and *N*-trimethoxysilylpropyl-*N,N,N*-tri-n-butylammonium chloride (TSPBC, 50% in methanol) were obtained from Gelest, Inc (Morrisville, PA, USA). Ethanol (200 proof) was obtained from the Warner-Graham Company (Cockeysville, MS, USA). Water was deionized to 18.2 MΩ-cm by a Millipore Milli Q UV-Plus water purification system.

2.1. Materials synthesis

Table 1 provides a summary of the sorbent materials utilized and their morphological characteristics. In general, synthesis involves establishment of morphology-directing surfactant micelles in solution followed by condensation of precursors around those micelles. The surfactant is then removed from the material leaving a porous structure to which further modification can be made. Below, specific syntheses for the various materials are detailed.

BTE- and DEB-based materials. Synthesis of the BTE- and DEB-based sorbents was based on a previously described approach [49–51] and began with dissolving TMB and P123 (1.9 g) in 0.1 M HNO3 with stirring at 60˚C. The stirring solution was cooled to room temperature and the silane mixture was added drop-wise. The reaction mixture was stirred until homogeneous and transferred to a culture tube which was sealed tightly and heated at 60˚C overnight (∼18 h). The tube was unsealed and the white gel was heated at 60˚C for 2 d and then 80˚C for 2 d. P123 was extracted by refluxing the monolith three times in ethanol for at least 12 h. A powdered product was collected by suction filtration, rinsed with ethanol and water, and dried at 100˚C. For MM5, 0.3 g TMB was used with 6 g 0.1 M HNO3. The total mol Si used was 7.84 mmol with 50:50 BTE:DEB. In the case of P10 and P5, 0.55 g TMB was used with 7.5 g 0.1 M HNO3. The silane mixture consisted of 15.7 mmol total Si with either 50:40:10 (P10) or 50:45:5 (P5) BTE:DEB:PTS. For N5, the protocol utilized 0.3 g TMB with 9.5 g 0.1 M HNO3 and the silane mixture was 100% BTE [49]. Following synthesis, amine groups were grafted on to the materials by adding sorbent (1 g) to 200 mL of toluene with 1 g APS [52]. This mixture was refluxed for 24 h after which the grafted product was collected by vacuum filtration, washed with toluene then ethanol, and dried at 110˚C. The DEN sorbent is a variation of this material. Following the amine functionalization protocol, isocyanate groups were incorporated using the ICS precursor [52]. This sorbent (1 g) was then placed in 50 mL MES buffer (100 mM, pH 5.5) with 1.3 g PAMAM dendrimer (10 wt. % in methanol) and mixed on a rotisserie mixer overnight at room temperature. The sorbent was collected by vacuum filtration, washed with methanol, and dried at 110˚C.

Sorbents with alkylammonium groups. The alkylammonium group bearing materials were synthesized based on a previously published approach [50, 51, 53]. For synthesis of the HX sorbent, 4.0 g of Pluronic P123 and 0.85 g of TMB were dissolved in 12.0 g of 1.0 M HNO3 with magnetic stirring and heating at 60˚C. The stirring mixture was allowed to cool to room temperature and 5.15 g of TMOS was added drop-wise. The mixture was stirred until homogeneous, transferred to a culture tube, sealed tightly, and heated at 60˚C overnight (≥18 h). The white monolith was dried in the unsealed tube at 60˚C for approximately 5 days before calcination (ambient atmosphere, temperature ramped 1˚C/min to 650˚C and held for 5 h) to

remove surfactant. Materials were dried at 110˚C prior to grafting with alkylammonium silanes which was accomplished by adding sorbent (1 g) to 100 mL of toluene followed by addition of 2 mmol of both TSPMC and TSPBC. This mixture was refluxed for 24 h after which the grafted product was collected by vacuum filtration, washed with toluene then ethanol, and dried at 110˚C. The CF2M2B sorbent was synthesized identically with the exception of the TMB included which was 3.10 g.

Metal functionalized sorbents. EDA materials were synthesized using an adapted protocol [54–56] in which BTE (3.2 g) was dissolved in 0.01 M HCl (4 g). P123 (0.65 g) was added to the mixture and allowed to fully dissolve. The metal chelating group, EDA (0.11 g) was then added with either zinc chloride (0.04 g) or copper chloride (0.04 g) and a vacuum was pulled on the solutions for 24 h. The tube was sealed and placed in an oven at 100˚C for 0.5 h followed by 60˚C for 24 h. Sorbents were refluxed twice in acidified ethanol to remove the surfactant and soaked overnight in an ammonium hydroxide solution. After rinsing, metals were reincorporated through refluxing in a 0.1 M solution of either copper chloride or zinc acetate.

Chitosan functionalized sorbent. The reactor for this synthesis consisted of a 1000 mL PTFE jar in a water bath maintained at 80˚C. Cetyltrimethylammonium bromide (1.0 g) and 1.0 N NaOH (6.0 mL) were dissolved in 475 mL of H2O with magnetic stirring [57, 58]. Mesitylene (6.0 mL) was added, and the solution was stirred for 3 h. Tetraethyl orthosilicate (TEOS; 5 mL) was added, and the mixture was stirred; white precipitate formed quickly. After 2 h, the precipitate was collected on filter paper by gravity filtration. When dry, the material was refluxed in 160 mL of ethanol with 9 mL of hydrochloric acid (37%) for 1 d to extract surfactant. The extracted product was collected by centrifugation, and washed with ethanol followed by water (3 times). The sorbent was dried at 110˚C prior to functionalization. To incorporate chitosan, a mixture of 1 g chitosan and 100 mL of 1 vol% acetic acid was prepared and filtered to remove insoluble matter. The sorbent was magnetically stirred in 50 mL of chitosan solution at room temperature for 1 d. The functionalized material (ChTS) was collected by centrifugation and washed with H2O three times before drying at 70˚C.

2.2. Characterization methods

Nitrogen sorption experiments were conducted using a Micromeritics ASAP 2010 at 77 K with DataMaster v. 4.03 E control and analysis software. Samples were degassed to 1 μm Hg at 100˚C prior to analysis. Standard methods were used for calculation of material characteristics. The Brunauer-Emmett-Teller (BET) method was used to determine surface area; Barrett-Joyner-Halenda (BJH) method was used to calculate pore size from the adsorption branch of the isotherm; the single point method was used calculate pore volume at relative pressure (P/P0) 0.97.

Powder X-ray diffraction patterns were collected at room temperature using CuKα radiation from a Brüker MICROSTAR-H X-ray generator operated at 40 kV and 20 mA equipped with a 5 m Radian collimator, and a Brüker Platinum-135 CCD area detector. A custom fabricated beamstop was mounted on the detector to allow data collection to approximately 0.4˚ 2θ (approximately 210 Å) with a sample to detector distance of 30 cm. After unwarping the images, the XRD2 plug-in was used to integrate the diffraction patterns from 0.3˚ to 8.4˚ 2θ.

2.3. Control nucleic acids

Triosephosphate isomerase (TIM, accession no. AF247559) of *Arabidopsis thaliana* was chosen as control RNA. TIM RNA transcripts were generated as previously described from pSP64poly(A)-TIM linearized with EcoRI and *in vitro* transcribed from the SP6 promoter using the MEGAscript high-yield transciption kit (formerly Life Technologies, Carlsbad, CA, USA; now ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's recommended protocol [58]. *A. thaliana* NAC1 (accession no. AF198054) was chosen as control DNA and a 1059 nucleotide segment of the gene was PCR out of cDNAs of *A. thaliana* and cloned into TOPO4.0 vector (Life Technologies, Carlsbad, CA). The plasmid containing the NAC1 gene was used as a template for PCR with M13 primers, then digested with restriction enzymes PstI and XbaI (New England BioLabs, Inc., Ipswich, MA, USA). This was then cloned into pSP64 polyA Vector (Promega Corporation, Madison, WI, USA) digested with the same enzymes to generate pSP64poly(A)-NAC. NAC1 DNA was amplified by PCR with SP6 and M13R primers, and the PCR products were purified using a QIAquick PCR purification kit (QIAGEN, Valencia, CA, USA).

Single strand NAC1 DNA (ssNAC1) was generated based on the protocol developed by Tang et al. [59] with slight modification. Briefly, ssNAC1 was PCR amplified in 50 μL reaction volume containing 1x Green GoTaq® reaction buffer (Promega Corporation, Madison, WI, USA), 3 mM MgCl2, 200 μM each of dNTPs, 20 nM each of SP6 and M13R primers, 375 nM of NAC1 forward primer, 1 unit of GoTaq® DNA polymerase (Promega Corporation, Madison, WI, USA), and 0.01 ng of pSP64poly(A)-NAC DNA. Reactions were performed with initial denaturation at 95˚C, 3 min, followed by 20 cycles of 95˚C, 20 sec; 52˚C, 30 sec; and 72˚C, 60 sec; and 20 cycles 95˚C, 20 sec; 58˚C, 30 sec; and 72˚C, 60 sec with final extension at 72˚C, 5 min. The ssNAC1 PCR products were confirmed using 1.5% TAE agarose gel, then purified using DNA clean & concentratorTM-5 (Zymo Research, Irvine, CA, USA) according to manufacturer's recommended protocol for ssDNA purification.

2.4. Nucleic acid adsorption and elution

Adsorption of nucleic acids (NAs) by the porous sorbents was performed as previously described [58]. Briefly, samples were vortexed, placed on an agitator, and incubated for 20 min. Following incubation, samples were centrifuged at 1,000 g for 10 min and supernatants were separated from the precipitated sorbents. Bound target was calculated by comparison of supernatant content as determined by PCR to that determined for the control solution. Generation of isotherms and fits of the NA binding data were completed using PSI-plot v. 9.5 (Poly Software International, Inc., Pearl River, NY, USA).

To wash sorbents prior to nucleic acid adsorption, 0.3 mg of sorbent in 300 μL nuclease-free water was centrifuged at 1,000 g for 10 min. The supernatant was removed, and the sorbent was resuspended in 300 μL of 10 mM Tris/1% Triton X-100 followed by incubation for 15 min at room temperature with agitation. The sorbents were again centrifuged at 1,000 g for 10 min, and the supernatant was aspirated and discarded. The sorbents were then resuspended in 330 μL of 10 mM Tris/1% Triton X-100 for use in adsorption experiments as described above.

NA elution was performed using 20 to 100 μL of different buffers at various temperatures as indicated in the text and figure captions. Sorbents with encapsulated NA were mixed with elution buffer and vortexed briefly, then incubated at the indicated temperature for 10 min. After incubation, the samples were centrifuged at 1,000 g for 10 min; the supernatants were used for quantitative real-time reverse transcription-PCR (qRT-PCR) or real-time PCR (qPCR). NA content was determined based on a comparison of the results of PCR analysis to that of control solutions.

For stability testing, supernatants were separated from the precipitated sorbents, and the sorbents were left to dry at room temperature overnight. Control target samples were stored as prepared for adsorption experiments (in solution). To collect a time point, elution was completed as described above. NAs recovered at various time points were compared to recovery of NAs from the same sorbent on day 1 of the experiment.

2.5. Real-time RT-PCR or PCR (qRT-PCR or qPCR)

The recovery rates of TIM RNA were quantified using qRT-PCR as previously described [58]. The recovery rates of NAC1 and ssNAC1 were quantified using qPCR. qPCR was performed using iQ SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) with primers NAC1-225F (5'-ATCGACCACCTCTTGTCCTG-3') and NAC1-377R (5'-CCGTTGCTCGG TTAGTTCTC-3'). Eluted NA (1 μL) was used as template for 25 μL PCR reactions using MyiQ Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). A serial dilution of 10x NAC1 DNA (1–105 fg/μL) was used as standard curve. The qPCR reactions were performed using the following conditions: 95°C for 3 min, followed by 30–35 cycles of 10 sec of denaturing at 95°C, 10 sec of annealing at 56°C, 10 sec of extension at 72°C.

3. Results and Discussion

The sorbents utilized for this effort offer a range of morphological and chemical functionalities (Table 1). Pore diameters for the materials range from 43 to 223 Å while the BET surface areas range from 140 to 1,000 m^2/g. Surface area, together with morphological organization, can impact the total binding sites available to an application. As the nucleic acid targets considered here are large molecules, average pore diameters and related factors, such as diffusion, may also contribute to the binding characteristics. While these factors cannot be ignored in overall sorbent optimization, this study did not focus on the impact of morphology; rather, the emphasis is given to the potential for the chemical composition of the materials in stabilizing the targets against typical degradation processes.

The chemical composition of the sorbents considered covers a wide range of possible activities (Figure 1). Diethylbenzene-bridged materials and those functionalized with pendent phenyl groups offer a somewhat hydrophobic environment as well as a high concentration of π-bonds (MM5, P5, P10). The hydroxyl groups of these types of silicate materials tend to be acidic; incorporation of primary amine groups offers basic sites (N5). The dendrimer modification (DEN) provides a greater number of basic sites at greater distance from the surface and increased hydrophobicity in the sorbent. The alkylammonium functionalities offer cationic groups in two different material morphologies with relatively disordered (CF2M2B) and ordered (HX2M2B)

Table 1: Material characteristics.

Material	Description	S. Area (m^2/g)	Pore Vol (cm^3/g)	Pore Dia (Å)
NS*	Bare silicate sorbent; no organic groups	730	0.75	50
N5	Primary amine groups on BTE sorbent	1002	1.19	77
P5	Phenyl groups on DEB sorbent	470	0.46	50
P10	Phenyl groups on DEB sorbent	440	0.43	43
MM5	DEB sorbent	606	0.51	44
CuEDA	Coordinated copper on BTE sorbent	716	0.87	64
ZnEDA	Coordinated zinc on BTE sorbent	275	0.70	223
HX2M2B	Alkylammonium groups on ordered pore structure (TMOS)	169	0.26	63
CF2M2B	Alkylammonium groups on mesostructure cellular foam (TMOS)	143	0.18	93
DEN	Amine and C12 terminated dendrimer on BTE sorbent	649	0.75	40
ChTS	Chitosan on TEOS	550	0.82	54

*NS is a TEOS-based sorbent from previous work [58].

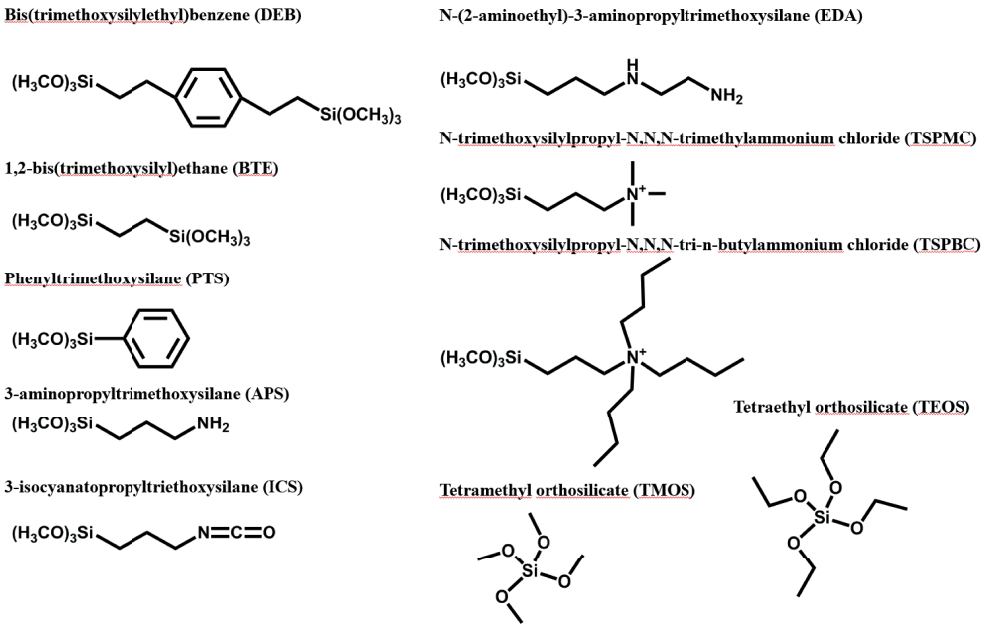

Figure 1: Precursors groups utilized in synthesis of sorbents.

mesopore structures. Chitosan (ChTS) offers antimicrobial activity as well as the potential for multiple and complex cationic interactions with nucleic acids. The materials with ethylenediamine pendent groups (CuEDA and ZnEDA) offer sites for metal ion chelation. The presence of cations is known to impact the secondary structure of DNA; the presence of copper has been shown to decrease DNA melting temperatures while zinc causes an increase [60].

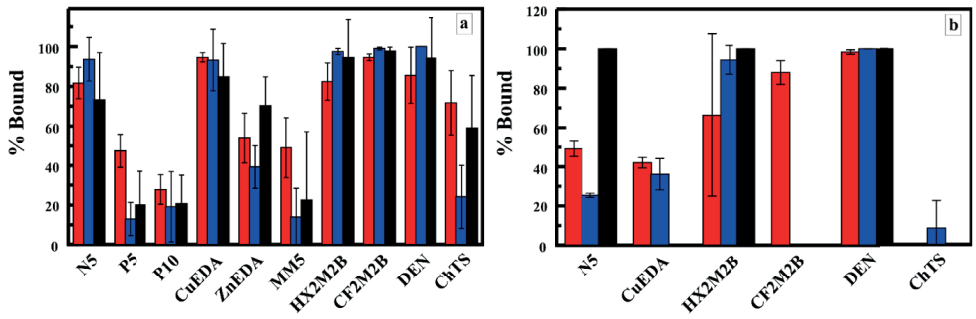

Figure 2: NA targets bound from solutions consisting of 30 μg sorbent with 300 ng of TIM RNA (red); 3 ng NAC1 DNA (blue); or 30 ng NAC1 ssDNA (black). Sorbents were used without pre-washing (a) and following the described wash steps (b). Error bars indicate standard deviation across six measurements. Data with * indicate those for which distinct differences in target binding was noted..

3.1. Target adsorption and elution

The materials were screened by generating a data set consisting of binding data for RNA and DNA using two target concentrations (Figure 2a). The goal was to identify the types of functional characteristics that provided significant adsorption of the two targets, so that those materials could be evaluated fully. The results of this screening were unexpected; some materials bound high percentages of the DNA or RNA while binding little to none of the other target (see items identified by asterisks). Similar data for the binding of ssDNA was collected to determine if the single versus double stranded nature of the targets was a significant contributor to these differences and to provide an additional point of comparison between the materials. The results of ssDNA binding were consistent with neither the DNA nor RNA binding (Figure 2a) indicating that the single or double stranded nature of the targets was not the primary contribution to the difference in binding efficiency of the materials.

The Langmuir-Freundlich (LF) binding isotherm is a generalized form of the phenomenological Langmuir model often applied to solid sorbents. It allows for calculation of an effective affinity constant for the target (k), the saturated loading capacity of the sorbent (q_s), and the site heterogeneity (n) within the sorbent based on the free ([L], ng) and bound target (q, ng/μg) [58, 61, 62]. Here, the constant α divided by the mass (m) yields the more typically utilized saturation capacity (q_s) for the model.

$$q = \frac{\frac{\alpha}{m} k[l]^n}{1 + k[l]^n} \tag{1}$$

In order to better understand the interactions between the NA targets and the sorbents, isotherms for each of the materials with each of the three targets were generated. Table 2 provides a summary of the resulting parameters and fit statistics. In the case of these materials, site heterogeneity was found to be one (n = 1) for all targets; this result was also observed for a previous RNA binding study [58]. Figure 3a presents data from RNA adsorption experiments and the calculated binding isotherms for N5. As in past studies of this type, adsorption of target using varied target and sorbent concentrations provides sampling across a larger region of the binding space and allows for generation of fit parameters with greater confidence [48].

The parameters obtained indicate the maximum target that can be bound (α) and the rate at which that limit will be approached (k). For example, the RNA saturated loading limit for

Table 2: Langmuir isotherm parameters for RNA, DNA, and ssDNA binding by unwashed sorbents.

	RNA				DNA				ssDNA			
Material	**α (ng/μg · μg)**	**k (ng^{-1}) $\times 10^{-3}$**	**Chi^2**	**St. Error**	**α (ng/μg · μg)**	**k (ng^{-1}) $\times 10^{-3}$**	**Chi^2**	**St. Error**	**α (ng/μg · μg)**	**k (ng^{-1}) $\times 10^{-3}$**	**Chi^2**	**St. Error**
Unwashed Sorbents												
NS*	410	550	—	—								
N5	165	46.7	381	3.45	1530	38.3	105	4.81	75.7	4.72	136	1.39
P5	204	6.91	691	3.91	9.14	0.661	187	6.34	62.6	0.512	25.7	0.607
P10	104	7.92	552	3.50	9.26	0.698	561	9.71	38.2	0.478	45.9	0.810
CuEDA	690	6.03	2920	7.09	118	8.53	852	4.33	147	1.12	407	2.41
ZnEDA	301	1.95	234	2.26	55.8	4.14	422	10.5	104	0.707	75.4	1.14
MM5	97.6	17.7	200	2.08	9.13	0.614	110	1.33	57.6	177	200	2.08
HX2M2B	375	8.18	1421	4.95	114	8.27	60.3	3.62	256	1.78	327	2.67
CF2M2B	571	9.18	3580	8.83	26.2	1.91	118	1.60	158	1.06	141	1.83
DEN	279	31.8	960	5.73	N/A†	N/A	N/A	N/A	94.8	0.563	634	3.71
ChTS	255	42.9	1520	5.75	N/A†	N/A	N/A	N/A	7.43	0.467	5.64	0.284
Washed Sorbents												
N5	300	6.13	124	1.64	61.4	4.48	1315	8.48	157	1.10	41.7	0.952
CuEDA	167	30.2	924	3.60	60.6	4.41	905	6.17	N/A†	N/A	N/A	N/A
HX2M2B	220	82.1	247	2.06	8.78	0563	344	11.1	113	0.728	16.4	0.604
DEN	165	202	1260	3.35	184	13.3	213	6.81	176	1.22	90.4	1.43

*NS is the base sorbent from previous work [58].

†Insufficient data for generation of an isotherm; DEN ~100% bound, ChTS ~0% bound, CuEDA. ~0% bound

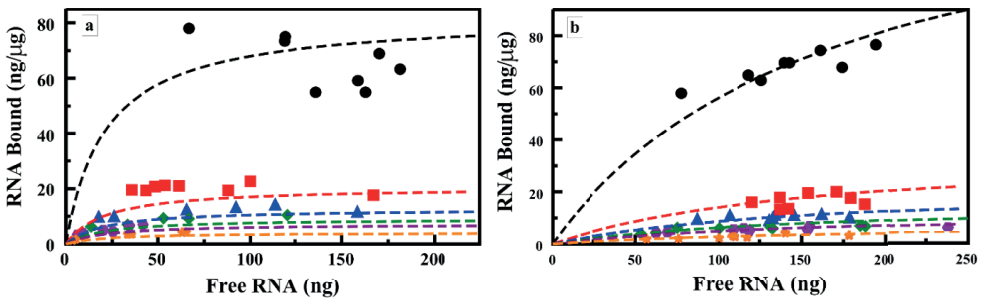

Figure 3: Binding Isotherms. RNA bound by the N5 sorbent without pre-washing (a) and following the described wash step (b). Here, sorbent masses of 2 (black circle), 8 (red square), 13 (blue triangle), 18 (green diamond), 23 (purple hexagon), and 40 μg (orange star) were utilized for capture of RNA from a 240 μl solution containing ~300 ng TIM RNA target.

HX2M2B is greater than that of N5, but, at low free RNA concentrations, N5 will bind more target than HX2M2B (k = 0.0467 ng^{-1} versus 0.00818 ng^{-1}). N5 provided the greatest DNA saturated loading limit, while HX2M2B provided the greatest limit for ssDNA. MM5, P5, and P10 showed moderate to low total binding and affinity for all three targets. DEN performed moderately for RNA and ssDNA and bound by far the greatest amount of DNA. CuEDA and ZnEDA performed moderately for DNA and ssDNA, but CuEDA provided the greatest RNA

saturated loading limit. Elution of bound target from the sorbent materials was also evaluated. Initial attempts at recovering RNA using EB buffer (10 mM Tris-Cl, pH 8.5) at 50˚C (total volume 20 μL) provided minimal return from these materials. NEB buffer (20 mM Tris-Cl, pH7.5, 1 mM EDTA) provided the best performance in our previous study [58], but did not offer target recovery from these materials. Variations on temperature, volume, incubation period, and detergent concentrations were considered and tested as was the inclusion of solvent and sodium chloride [63, 64]. Other studies have indicated the impact of buffer pH on the elution efficiency related to silicate materials [65–67]; however, varying pH (5.7 to 8.0) did not have an impact on RNA recovery. It has been argued that nucleic acid interactions with silicate materials are via amine and carboxyl groups [68]. Methods used to displace these interactions as well as those used to displace RNA from negatively charged membranes [69] were considered and tested without improvement. Finally, various nucleic acid washing solutions and hybridization buffers were evaluated without success (less than 1% of target recovered). The solutions and condition variations evaluated are summarized in Table 3.

Table 3: Elution solutions and conditions evaluated.

Solution	Volume (μL)	Time (h)	Temperature (˚C)
EB buffer (10 mM Tris-Cl, pH 8.5)	20, 50, 200	0.3, 3	50
EB buffer (10 mM Tris-Cl, pH 8.5) with 0.1% Tween 20	50	0.3	50
EB buffer (10 mM Tris-Cl, pH 8.5) with 0.1% SDS	50	03	50
EB buffer (10 mM Tris-Cl, pH 8.5) with 0.1% SDS and Tween20	50	0.3	50
Nuclease-free water	20, 50, 200	0.3, 3	RT, 65, 95
1x GoTaq PCR buffer (Promega) with 0.1% SDS	20, 100, 200	0.3, 3	50
NEB buffer	50	0.3	50
NEB buffer with 0.1% SDS	50, 100	0.3	50
50 mM sodium phosphate buffer pH 5.7 to 8.0	50	0.3, 5	50
50 mM sodium phosphate buffer with 0.1% Tween 20, pH7.2	50	0.3	50
10, 50, 100, 200, or 250 mM Tris pH 8.0	50, 100, 200	0.3	50
10, 100, or 200 mM Tris with 20% ethanol	100	0.3	50
10 mM Tris with 50, 100, 150, or 200 mM NaCl pH 8.0	50, 100, 200	0.3	50
10 mM Tris with 1, 5, or 10% Triton X100 pH 8.0	50	0.3	50
10 mM Tris with 100 mM NaCl and 0.1% SDS	100	0.3	50
10 mM Tris with 100 mM NaCl and 1% Triton X-100	100	0.3	50
25 mM Tris with 250 mM Glycine pH 8.0 or 7.0	100	0.3	50
25 mM Tris with 250 mM Glycine & 0.1% SDS pH 8.0 or 7.0	100	0.3	50
25 mM Tris with 250 mM Glycine & 1% Triton X-100	100	0.3	50
100 mM Tris with 50 mM Glycine pH 8.0 or pH 9.5	100	0.3	50
100 mM Tris with 50 mM Glycine and 0.1% SDS pH 9.5	100	0.3	50
100 mM Tris with 50 mM Glycine and 1% Triton X-100 pH 8.0	100	0.3	50
100 or 200 mM Tris with 0.1% SDS pH 8.0	100	0.3	50
200 mM Tris with 50 or 100 mM NaCl pH 8.0	50, 100	0.3	50
200 mM Tris with 100 mM NaCl and 20% ethanol, pH8.0	100	0.3	50
200 mM Tris with 0.1% SDS and 20% ethanol, pH 8.0	100	0.3	50
200 mM Tris with 0.1% SDS, pH 8.0	100	0.3	50
Hyb buffer (MiSeq)	100	0.3	50
Hyb buffer (Affymetrix)	100	0.3	50

Solution	Volume (μL)	Time (h)	Temperature (°C)
0.2X or 2X SSC with 0.1% SDS	100	0.3	50
0.6X or 6X SSPE with 0.1% SDS	100	0.3	50
0.31, 0.63, 1.3, or 2.5 M NaCl	100	0.3	50
50 mM Glycine with 150 mM NaCl pH 9.5	100	0.3	50
50 mM Glycine with 150 mM NaCl and 0.1% SDS pH 9.5	100	0.3	50
1xTAE with 0.1%SDS	100	0.3	50
Washed Sorbents			
10 mM Tris pH 8.0	100	0.3	50
10 mM Tris with 20% ethanol	100	0.3	50
100 mM Tris with 0.1% SDS	100	0.3	50
10 mM Tris with 100 mM NaCl	100	0.3	50
NEB with 0.1% SDS	100	0.3	50
Washed DEN Sorbent			
1x PCR 0.1% SDS	100	0.3	50
NEB with 0.1% SDS			
10 mM Tris pH 8.0	100	0.3	50
10 mM Tris with 20% ethanol	100	0.3	50
10 mM Tris with 100 mM NaCl	100	0.3	50
10 mM Tris with 100 mM NaCl and 0.1% SDS	100	0.3	50
10 mM Tris with 100 mM NaCl and 1% Triton X-100	100	0.3	50
25 mM Tris with 250 mM Glycine and 0.1% SDS	100	0.3	50
25 mM Tris with 250 mM Glycine and 1% Triton X-100	100	0.3	50
100 mM Tris with 0.1% SDS	100	0.3	50
100 mM Tris with 50 mM Glycine and 0.1% SDS	100	0.3	50
100 mM Tris with 50 mM Glycine and 1% Triton X-100	100	0.3	50
0.6X SSPE with 0.1% SDS	100	0.3	50
0.6X SSPE with 1% Triton X-100	100	0.3	50

Given the failure of this wide range of elution solutions, the possibility that the nucleic acids were destroyed upon interaction with the sorbents was considered; however, given previous work in this area and the wide range of sorbent variations involved, these types of destructive interactions are unlikely to be observed for all of the materials under consideration. Based on previous experience and other materials used for nucleic acid hybridization, a pre-wash step was evaluated for the sorbents. This type of prehybridization step has been used in Northern and Southern blotting technologies to reduce nonspecific binding of nucleic acids [70]. Here, the procedure involved incubating the sorbent in 10 mM Tris with 1% Triton X-100 for 15 min at room temperature prior to NA adsorption. Figure 2b provides single point data on target binding by the washed N5, CuEDA, HX2M2B, CF2M2B, DEN and ChTS. Other sorbents considered for this study bound less than 5% of all three targets. This pre-conditioning step strongly impacted the binding behavior of the sorbents and led to less error in the resulting fits (Table 2; Figure 3b). ZnEDA offered lower saturated loading capacities than CuEDA prior to washing and likely lost binding capacity upon interaction with the Triton X-100 as observed for CuEDA. This surfactant would also be expected to interact with the surfaces of the MM5, P5, and P10 sorbents given their somewhat hydrophobic nature and the available π-interaction sites. Additional experiments using other prehybridization solutions, such as 2X SSC (0.3 M sodium chloride with 30 mM

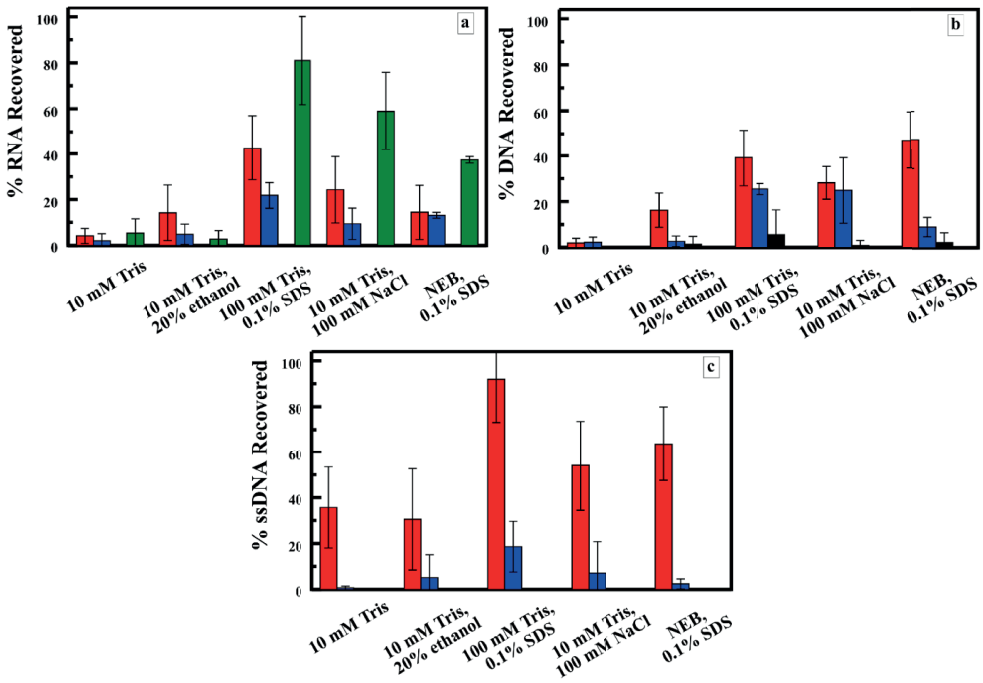

Figure 4: Elution. RNA eluted from washed sorbent materials reported as a percentage of the target initially adsorbed: N5 (red); HX2M2B (blue); DEN (black); CuEDA (green). All elution solutions utilized 100 μL at 50°C for 20 min: RNA (a), DNA (b), and ssDNA (c).

trisodium citrate at pH 7) with 0.1% SDS or 6X SSPE (900 mM NaCl with 60 mM sodium phosphate and 6 mM ethylenediaminetetraacetic acid) with 0.1% SDS, could be considered for use with these sorbents.

The potential for elution of RNA was evaluated using the pre-conditioned materials, and variations on the elution solution were again considered (Table 3). As shown in Figure 4a, RNA recovery was significantly increased from less than 1% in the unwashed sorbents to between 20 and 80% using 100 mM Tris with 0.1% SDS for HX2M2B, N5, and CuEDA. Unfortunately, prewashing did not significantly improve recovery from DEN; a wide range of elution solutions were again considered with no recovery greater than 2% (Table 3). It is unclear if this is a result of continuing strong interactions between DEN and the NA targets or target degradation. Recovery of DNA and ssDNA was also improved following the pre-conditioning step with a small amount of DNA recovered from even the DEN sorbent.

3.2. NA stability

In order to evaluate the potential of the sorbents to enhance the stability of stored NA targets, a large batch sample for each target adsorbed onto each material was prepared. The sample was then divided into aliquots, the supernatants were separated from the precipitated sorbents, and the sorbents were left to dry at room temperature overnight. A control sample consisting of the target only in solution was retained for comparison. The sorbents were sampled following storage either at room temperature or at 37°C. No special protection from light or control of humidity was employed. Storage at 37°C is intended to simulate temperatures relevant to those expected at the high end of operational conditions. Over the course of the experiments, room

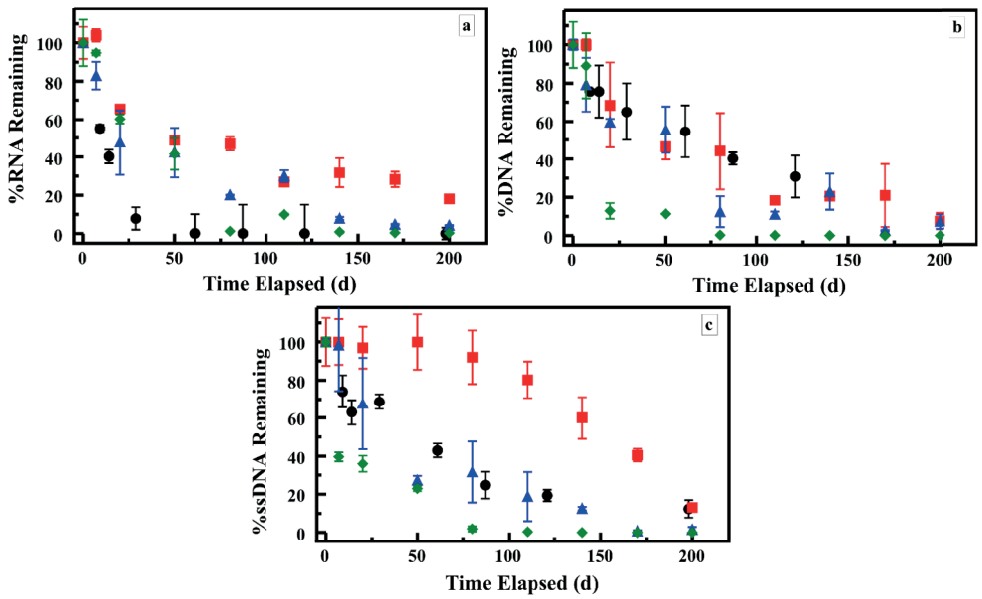

Figure 5: Stability of NA targets. NA targets recovered from N5 (red square), HX2M2B (blue triangle), and DEN (green diamond) following storage at room temperature. Data for similarly stored target only in solution (circle) is provided for comparison: RNA (a), DNA (b), and ssDNA (c).

temperature varied between 18 and 23˚C while relative humidity ranged from 42 to 61%. Figure 5a presents the results of RNA storage over a period of 200 days at room temperature. The recovered RNA is normalized to the amount recovered on day one of the experiment. Over this period, RNA eluted from N5 gradually decreases to 20% of the day one recovery while that from HX2M2B decreases to 5%. Recovery from DEN, significantly lower on day one, decreases to <10% by day 80. RNA in the control sample drops to <10% by day 29. At 37˚C, recovery of RNA from all three materials was increased as compared to that from the control sample (Figure 6a). More than 20% was recovered from N5 through day 140, from HX2M2B through day 80, and from DEN through day 50. These results indicate that the three sorbents offer improvements in RNA stability both at room temperature and at 37˚C.

The decrease in DNA recovered from N5 and HX2M2B at room temperature and at 37˚C was similar to the decrease in the DNA content of the control sample (Figure 5b and Figure 6b). For ssDNA, on the other hand, while the control sample at room temperature dropped below 20% of the original content on day 121, recovery from N5 remained above 20% beyond day 170. When stored at 37˚C, however, the decrease in ssDNA recovered from N5 was similar to that of the control sample. While the decrease in ssDNA recovered from HX2M2B at room temperature was similar to the decrease in the ssDNA content of the control sample, HX2M2B showed slightly improved recovery of ssDNA over the first 20 days at 37˚C. Results with DEN showed more rapidly decreasing DNA and ssDNA content than that observed for the control samples.

4. Conclusions

The study presented here evaluated the potential of mesoporous sorbents bearing chemical functionalities for stabilization of nucleic acid targets. The idea was to access the activities of

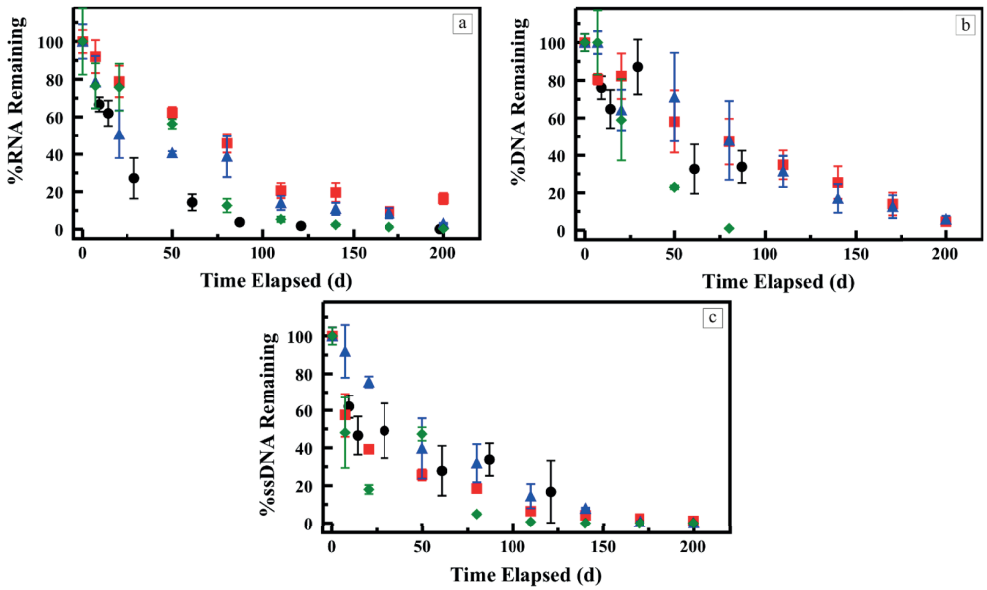

Figure 6: Stability of NA targets at 37°C. NA targets recovered from N5 (red square), HX2M2B (blue triangle), and DEN (green diamond) following storage at 37°C. Data for similarly stored target only in solution (circle) is provided for comparison: RNA (a), DNA (b), and ssDNA (c).

common stabilization reagents within these sorbents while making separation of the NAs from stabilizing compounds simple. This would allow for stabilization of the NAs during storage and transportation while providing a system that does not interfere with further (down-stream) analysis of the targets. While many of the porous materials were found to remove RNA, DNA, and ssDNA from solution, recovery of the targets from the sorbents proved challenging. The desire to avoid downstream contamination prevents the use of harsher elution conditions. The necessary pre-conditioning step using a Triton X-100 wash prevented the types of interactions desired with several of the surfaces considered. It is likely that π-interactions contributed to the noted reductions in binding for DEB based sorbents. Other commonly used prehybridization washing solutions, such as 2X SSC with 0.1% SDS or 6X SSPE with 0.1% SDS, could offer substitutions that reduce interaction with the π-bonds of the sorbents and are currently being evaluated.

Of the materials that were suitable for use with the pre-conditioning wash step, the sorbent functionalized with primary amine groups (N5) showed promising results for RNA and ssDNA stabilization. The performance of the sorbent at room temperature was approximately equivalent to the performance of our previously reported trehalose sorbent at 4°C [58]. N5 showed significant stabilization of RNA even when stored at 37°C. The HX2M2B sorbent also showed stabilization of RNA and ssDNA, but recovery from this sorbent declined more quickly than that of the N5 sorbent. None of the sorbents improved the stability of DNA either under room temperature or 37°C storage. Based on the results of these studies, we are currently evaluating the potential for combinations of functional groups within the sorbent structure. Specifically, combinations of sugars (trehalose, glucosamine) and the functional groups considered here within sorbent morphologies similar to those described for this study.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

This research was sponsored by the U.S. Office of Naval Research through Naval Research Laboratory base funds (69-6595). Participation of M. McConner through Office of Naval Research (ONR) sponsored Historically Black Colleges and Universities/Minority Institutions (*HBCU/MI*) summer internship program. Participation of G. Haas through Naval Research Laboratory Student Temporary Employment Program (STEP). We applied the SDC approach ("sequence-determines-credit") for determining the sequence of authors [71]. The views expressed here are those of the authors and do not represent those of the U.S. Navy, the U.S. Department of Defense, or the U.S. Government.

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