

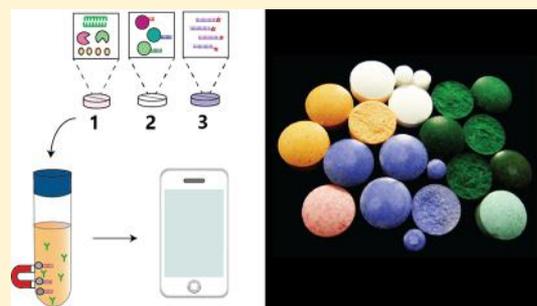
Simplifying Assays by Tableting Reagents

Buddhisha Udugama,^{†,‡} Pranav Kadhiresan,^{†,‡} Amila Samarakoon,^{†,‡} and Warren C. W. Chan^{*,†,‡,§,||,⊥,ⓑ}

[†]Institute of Biomaterials and Biomedical Engineering, [‡]Terrence Donnelly Centre for Cellular and Biomolecular Research, [§]Department of Chemistry, ^{||}Department of Chemical Engineering, and [⊥]Department of Materials Science and Engineering, University of Toronto, 160 College Street, Toronto, Ontario, Canada M5S 3E1

Supporting Information

ABSTRACT: Medical diagnostic assays provide exquisite sensitivity and precision in the diagnoses of patients. However, these technologies often require multiple steps, skilled technicians, and facilities to store heat-sensitive reagents. Here, we developed a high-throughput compression method to incorporate different assay components into color-coded tablets. With our technique, premeasured quantities of reagents can be encapsulated in compressed tablets. We show that tableting stabilizes heat-sensitive reagents and simplifies a broad range of assays, including isothermal nucleic acid amplification techniques, enzyme-based immunoassays, and microbead diagnostics. To test the clinical readiness of this tableting technology, we show the ability of tableted diagnostics for screening hepatitis B-positive patient samples. Our development simplifies complicated assays and the transportation of reagents and mitigates the need for refrigeration of reagents. This advances the use of complex assays in remote areas with limited infrastructure.



INTRODUCTION

Chemical assays that detect contaminants, small molecules, and biomarkers form the cornerstone of a number of fields, including environmental, food, and medical testing.¹ These assays require skilled technicians to accurately weigh, pipet, and resuspend reagents, accompanied by instrumentation and technical knowledge to plan and carry out the assays.^{2,3} The need for skilled personnel and instrumentation is compounded in medical diagnostic workflows where multiple assays may be required for a single diagnostic result.^{4,5} However, these requirements prevent the translation and accessibility of medical diagnostics in resource-limited areas with high burden of diseases and low infrastructural capabilities, leading to higher mortality and morbidity rates.⁶ In addition, these chemical assays use reagents, such as antibodies, small molecules, fluorescent probes, and nanoparticles, that can degrade or aggregate during shipping, transport, and storage at elevated temperatures.^{7,8} The thermal instability of such reagents can further compromise test results⁷ and lead to misdiagnosis of patients. By addressing these barriers, there is a unique opportunity to improve the accessibility of medical diagnostics in resource-limited settings at the point-of-care. Here, we developed a simple, cost-effective, and high-throughput compression strategy to make tablets that can simplify and thermally stabilize components of a diagnostic assay.

Tablets can simplify the diagnostic process while providing an inexpensive avenue to circumvent the thermal instability of reagents. In addition to simplifying complex diagnostic tests, premeasured quantities of reagents can be prepackaged into tablets to improve the reproducibility of assays. Tablets have high production speeds, they are easy-to-handle, and they have tunable dissolution times. Color-coded tablets can also be

developed to further simplify the diagnostic process by ensuring only minimal instructions are required for end users. In addition to being easy to use, tablets provide thermal stability to diagnostic reagents by encapsulating them in a compact matrix of chemical preservatives. The pore sizes within tablets can also be varied to minimize reagent contact with water vapor.⁹ High water content at elevated temperatures and humidity can facilitate the degradation of reagents either via hydrolysis or through increased molecular mobility and reactivity.¹⁰ By encapsulating reagents in a platform that minimizes interaction with water, reagents can be stabilized at elevated temperatures. One study used the film-forming properties of the polysaccharide pullulan to create stable reagent tablets for bioassays,¹¹ but this method was time-consuming and had low production speeds (number of tablets developed per minute). In addition, it was also unclear whether pullulan tablets protect reagents for a prolonged time at elevated temperatures.^{11,12} We developed compressed tablets to mitigate the issue of thermal instability of commonly used diagnostic reagents. Our method involves direct compaction of a mixture of powder blends, consisting of the active ingredient and excipients (or additives), to produce uniform tablets.¹³ Compressed tablets provide high dissolution rates, improved shelf-lives, and high production speeds.¹⁴

RESULTS

Principle of Chemical Interactions between Excipients (or Additives) and Diagnostic Reagents. Diagnostic reagents in solution can be subjected to hydrolysis, oxidation,

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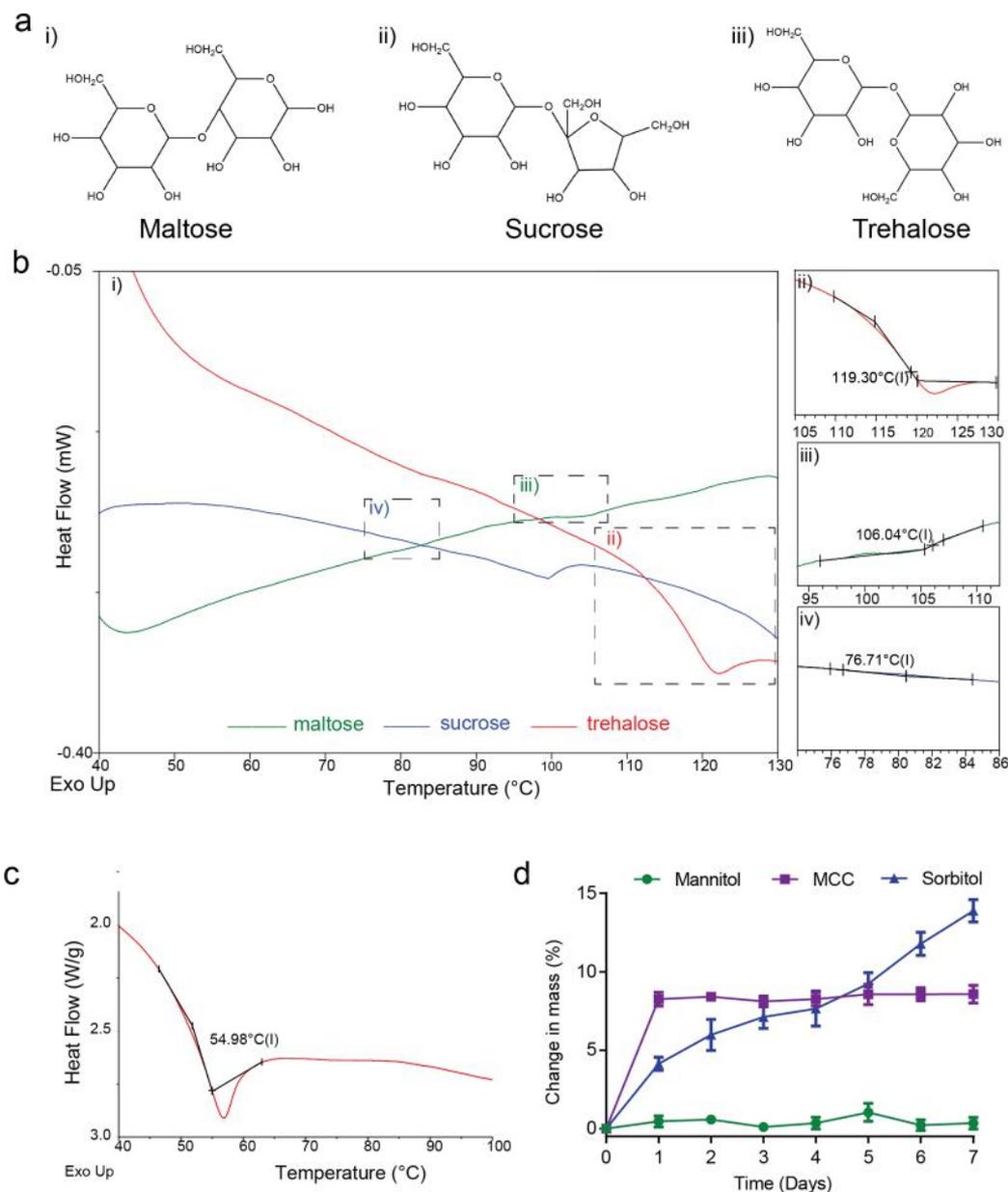


Figure 1. Screening chemical compounds for the development of reagent tablets. (a) Chemical structures of the sugars (i) maltose, (ii) sucrose, and (iii) trehalose used to prevent stress-induced biomolecular degradation during lyophilization. (b) (i) Differential Scanning Calorimetry (DSC) curves of heat flux vs temperature for trehalose, maltose, and sucrose. (ii) Inset with the T_g of trehalose. (iii) Inset with the T_g of maltose. (iv) Inset with the T_g of sucrose. (c) T_g after moisture uptake. (d) Weight changes of tablets stored at 80% room humidity (RH) for 1 week. Exo up: In heat flux DSC, exothermic events are recorded as peaks pointing upwards.

and other forms of degradations due to their high molecular mobility in water.¹⁵ The molecular mobility of reagents can be minimized by removing the surrounding water content via lyophilization. Although lyophilization has the potential to stabilize labile reagents, the process can generate excessive stresses that can further degrade diagnostic reagents.¹⁶ Sugars can be added to stabilize the reagents during lyophilization, although the selection of the type of sugar molecule is critical to this stabilization process. Sugars are postulated to protect biomolecules during lyophilization by forming a glassy matrix with low molecular mobility.¹⁶ However, there are a multitude of sugar molecules that can act to serve our purpose. For optimal stabilization, we wanted to select a sugar with a high glass transition temperature (T_g). The plasticizing effects of water can lower the T_g values of sugars during shipping and storage of

reagents at high humidity, leading to increased molecular mobility and consequently reagent degradation. Thus, we analyzed the T_g values of commonly used sugars, maltose, sucrose, and trehalose (Figure 1a), using differential scanning calorimetry (DSC) to determine the optimal stabilizer). We selected trehalose to stabilize reagents during lyophilization, owing to its high T_g (Figure 1b, graphs i–iv). Even after moisture uptake, the glass transition temperature of trehalose remained above the storage temperature of the reagents, allowing the reagents to have minimal mobility (Figure 1c). In addition, among the disaccharides, trehalose has the fewest intramolecular hydrogen bonds. Intramolecular hydrogen bonds restrict the movements of the ring structures in polysaccharides. The rigidity of polysaccharides can limit hydrogen bonding between the remaining hydroxyl groups and polar groups of biomolecules.

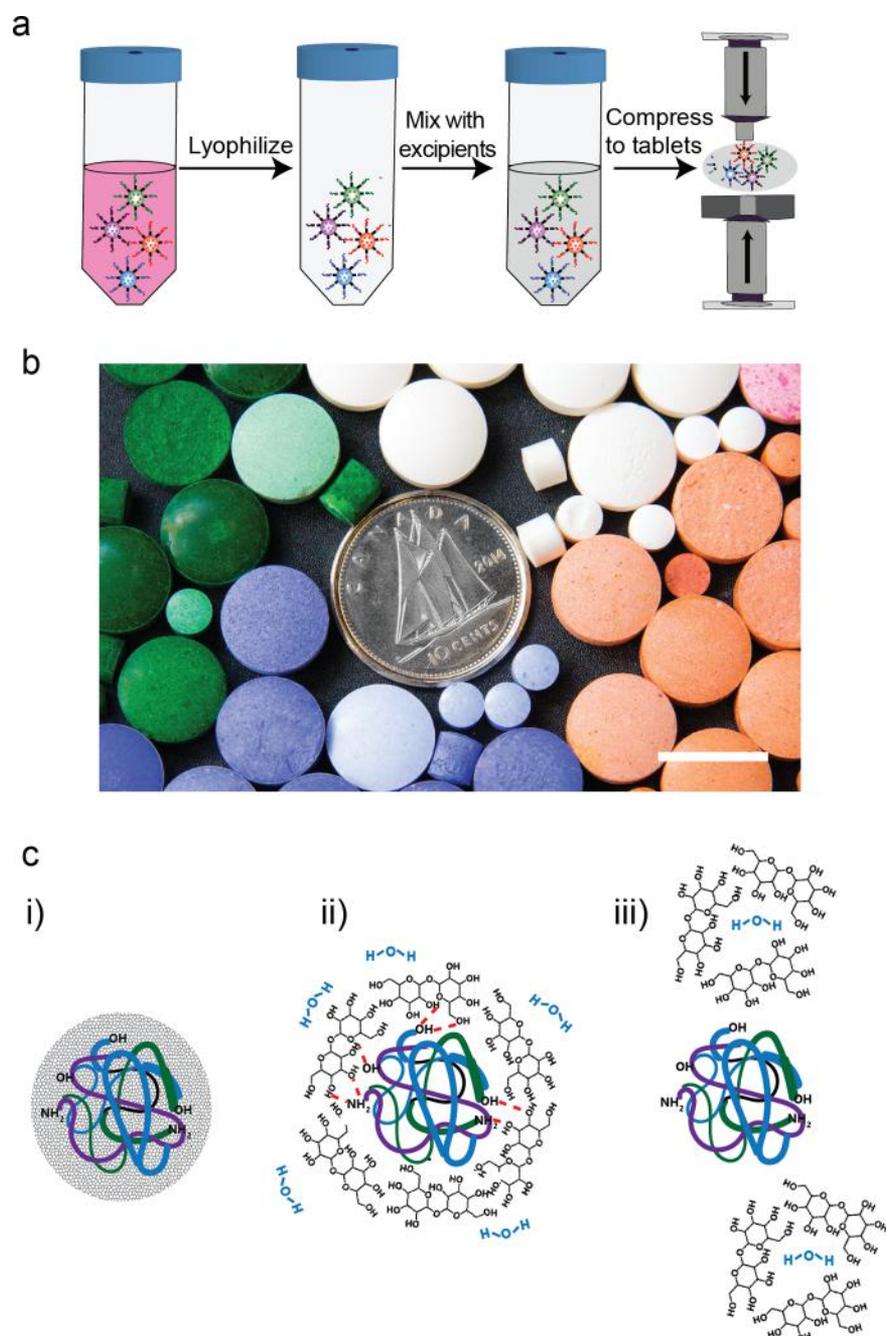


Figure 2. Development of compressed tablets. (a) Schematic representation of the development of diagnostic tablets. (b) Photograph of compressed tablets of various sizes and colors (scale bar 1 cm). Compressed tablets are inexpensive to develop, and the sizes and colors of the tablets can be customized for different types of assays. (c) Reagents are protected in tablets by using the stabilization properties of trehalose and the barrier environment created by the tablets. Trehalose is postulated to stabilize reagents (i) by reducing their mobility in a glassy matrix, (ii) by hydrogen bonding with the reagents and acting as a substitute for water, or (iii) by sequestering water and thereby reducing their interaction with water.¹⁹

The molecular flexibility of trehalose, on the other hand, allows it to replace the hydration shell around biomolecules, preserving the three-dimensional conformations of the reagents.¹⁷ Lastly, trehalose is a kosmotrope that disrupts the tetrahedral hydrogen-bonded network of water.¹⁸ Trehalose has a larger hydration number (the average number of water hydrogen bonded to the disaccharide), which allows water to be ordered around trehalose instead of the biomolecules.¹⁸ The “destructuring” of water molecules around biomolecules minimizes ice crystallization during lyophilization, thereby protecting the biomolecules from damage.^{18,19}

Next, to directly compress the reagents into easy-to-handle tablets, the reagents needed to be mixed with highly compactible powders. Thus, we screened spray-dried mannitol, D-sorbitol, and microcrystalline cellulose (MCC) to act as the bulking agents for the reagent tablets. To maintain the T_g of trehalose and to provide stability to the reagents, the bulking agent needed to be inert and provide a moisture-free environment. We stored tablets developed from spray-dried mannitol, D-sorbitol, and MCC for 1 week at 80% relative humidity (RH) and examined the changes in mass due to moisture uptake (Figure 1d). Among the three excipients, sorbitol had the greatest change in mass due to

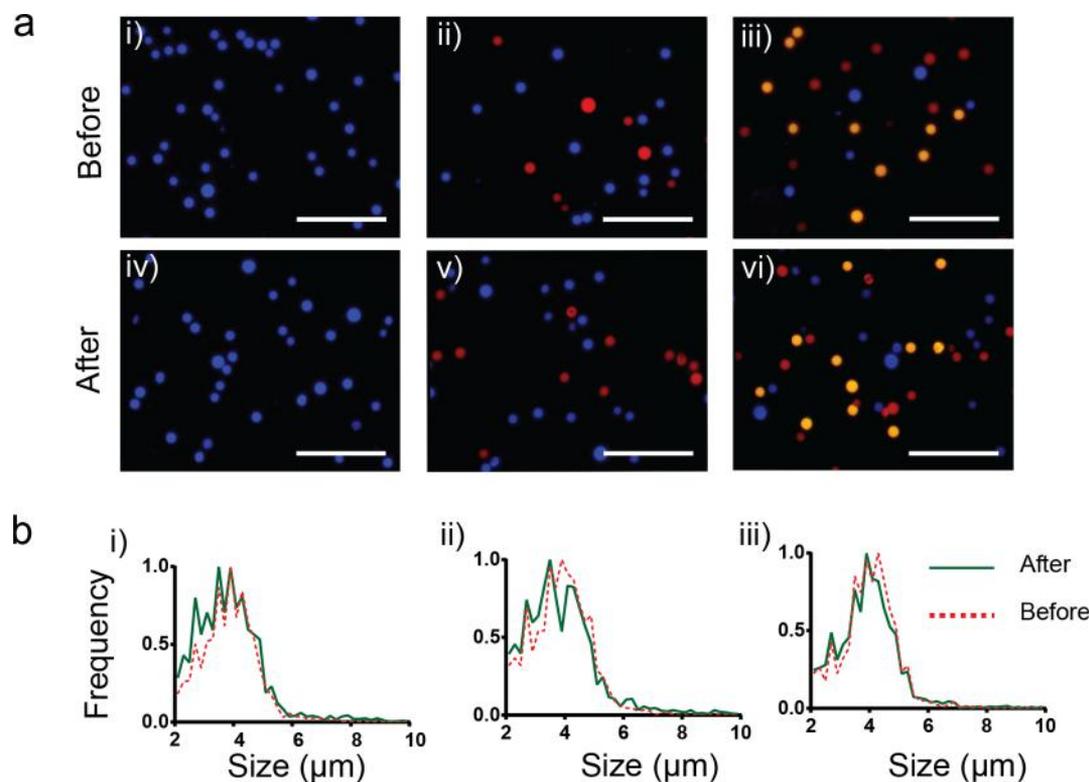


Figure 3. Characterization of reagent tablets using quantum dot barcodes as a model diagnostic system. (a) Fluorescence microscopy images of barcodes before and after encapsulation in compressed tablets (scale bar 50 μm). The positions of the microbeads were determined using 405/20 nm bandpass (BP) and 420 longpass (LP) excitation and emission filters. (i, iv) Fluorescence images before and after encapsulation of 3.9 μm 435 nm QD microbeads in compressed tablets. Images were obtained using 360/40 nm BP excitation and 447/60 nm BP emission filters (blue filter set). (ii, v) Composite images for two types of microbeads (435 and 586 nm) before and after encapsulation in compressed tablets. Microbeads were then imaged with both the blue filter set and 480/40 nm BP excitation and 650/50 nm BP emission (red filter set). (iii, vi) Composite images of three types of microbeads (435, 586, and 525 and 575 nm) before and after encapsulation in compressed tablets. Microbeads were imaged with the blue and red filter sets as well as with 480/40 nm BP excitation and 580/10 nm BP emission filter sets. (b) Size distribution of respective microbeads before and after encapsulation in compressed tablets.

moisture uptake. Although sorbitol is a diastereomer of mannitol, it is highly hygroscopic and absorbs moisture from the environment. The significant difference in hydration between the two isomers is attributed to the intermolecular forces in the solid state.²⁰ Unlike mannitol, sorbitol is able to form stable cocrystals with water at ambient conditions. The stability of the sorbitol–water cocrystals is due to a combination of both favorable molecular conformations and intermolecular cohesion relative to those of anhydrous sorbitol. Although MCC also exhibited low moisture absorption relative to sorbitol, reactive impurities found in MCC, such as trace levels of glucose, can react with amino groups of proteins in a Maillard reaction. In a typical reaction, the glycosidic hydroxyl group of glucose is replaced by an amine, producing glycosamine and other byproducts.²¹ Therefore, we selected spray-dried mannitol as the bulking agent of reagent tablets, owing to its inertness and nonhygroscopicity (Figure 1d).

Development of Reagent Tablets. Tablets were developed by first lyophilizing the diagnostic reagents, followed by mixing with additives and last compressing the mixture into individual tablets (Figure 2a). We first removed the surrounding water content by lyophilizing the reagents with 15% trehalose in 0.05% Tween 20. Trehalose, a nonhygroscopic cryoprotectant, provides a highly viscous glassy matrix to limit molecular mobility and stabilizes the reagents through hydrogen bonding.^{16,22} After lyophilization, we mixed the reagents with three excipients: spray-dried mannitol, croscarmellose sodium, and sodium stearyl

fumarate. We used spray-dried mannitol, a nonhygroscopic sugar–alcohol, as the bulking agent of the tablet.^{23,24} We also included croscarmellose sodium to help disintegrate the tablet and sodium stearyl fumarate to help lubricate the rotary press die walls for ease of tablet ejection.^{25,26} After mixing the reagents and excipients, we compressed the mixture with a rotary tablet press using a compression force of 800 N. We can generate customizable and readily dissolvable tablets (<30 s) at a rate of more than 300 tablets per minute (Figure 2b). In addition, the tablets can stabilize reagents by using the vitrification properties of trehalose as well as by providing a moisture-free barrier environment (Figure 2c).

Characterizing Reagents in Tablets Using a Model System: Quantum Dot (QD) Barcodes. To characterize the properties of diagnostic reagents in tablets, we used a multistep diagnostic assay. This assay uses QD barcodes, polymer microspheres encapsulated with QDs, that can detect a multitude of molecules in biological fluids.²⁷ QD barcodes can be characterized with multiple techniques, including fluorescence microscopy and flow cytometry, allowing for the ease of characterizing reagents in tablets. In a typical assay, multiple reagents, including the buffer, fluorescent detection probes, and target DNA, are all mixed with DNA-conjugated QD barcodes. The DNA-conjugated polymer microspheres, target DNA, and fluorescent detection probe form a sandwich complex. The signal from the fluorescent DNA probes and QD barcodes can then be detected to identify the presence and type of the target,

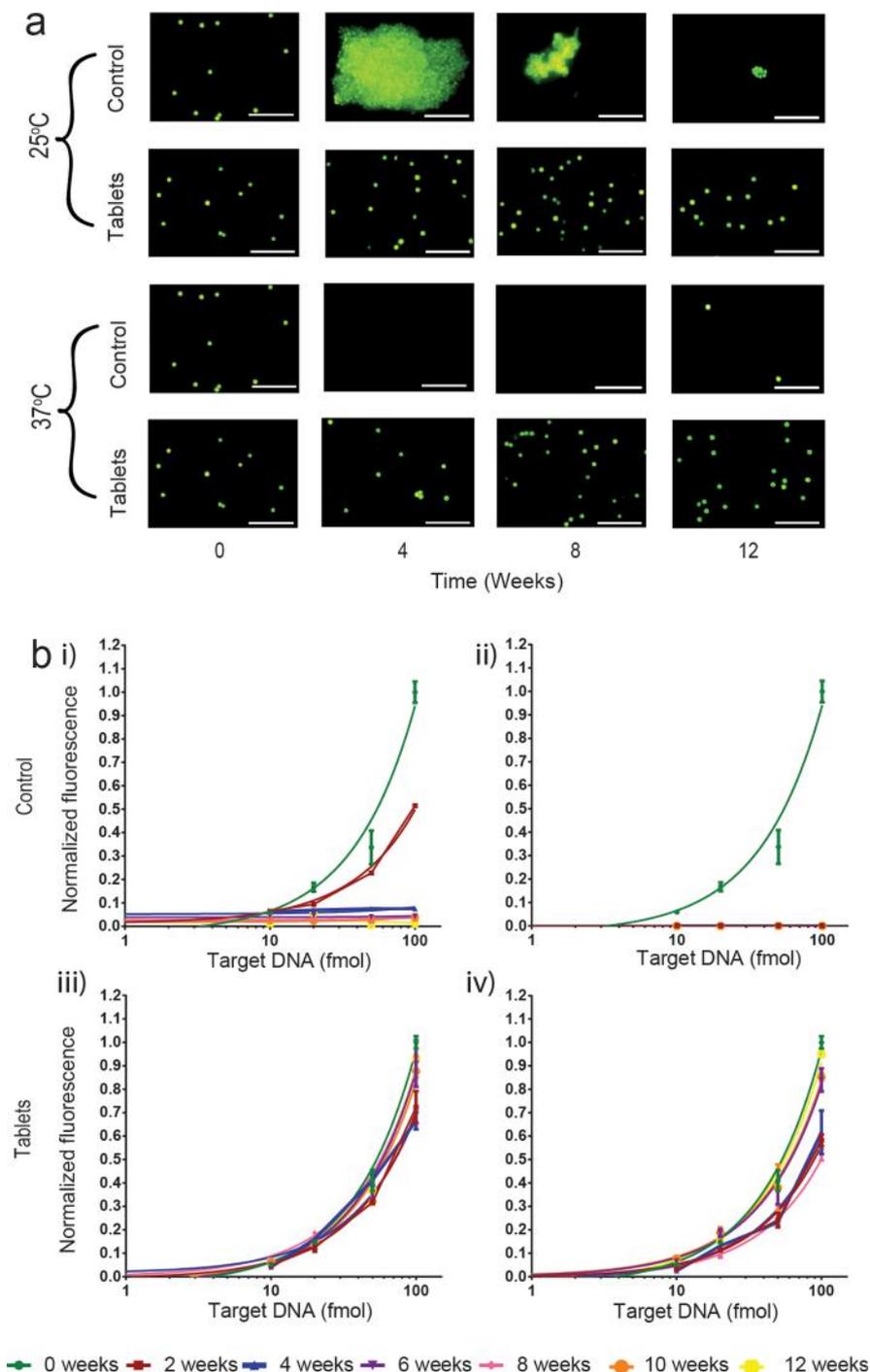


Figure 4. Characterization of thermal stability for barcodes in solution (control) and in tablets via a single-plex sandwich assay. (a) Fluorescence microscopy images (excitation 480/40 nm BP and emission 580/10 nm BP) of barcodes in tablets and in solution (control) for 12 weeks at 4 week time points. Over time, QD microbeads in solution aggregate as increased temperature leads to the disruption of noncovalent forces that hold the polystyrene copolymer of the microbeads. (b) Time-based sensitivity curves for barcodes in solution at (i) 25 °C and (ii) 37 °C and barcodes in tablets at (iii) 25 °C and (iv) 37 °C for 12 weeks at 2 week time points.

respectively.²⁸ We developed QD barcode tablets to first characterize the physical properties of diagnostic reagents post-tableting. Overall, when barcodes were encapsulated in compressed tablets, their structure, size, and fluorescence intensity were maintained (Figure 3). The process of tableting therefore does not change the physical properties of the reagents.

Characterizing the Thermal Stability of Reagent Tablets Using QD Barcodes. Next, we evaluated the thermal stability of the barcode assay components. We compared the

fluorescence, analytical performance, and structure of barcodes after storage in solution or tablets at various temperatures for 12 weeks (Figures S1–S3 and Tables S1 and S2, Supporting Information). QD barcodes in tablets and in solution were stored at 25 and 37 °C to mimic storing diagnostic reagents at room temperature and tropical climates.²⁹ QD barcodes stored in solution aggregated within 4 weeks at 25 °C and completely degraded within 2 weeks at 37 °C (Figure 4a). These properties impact the performance of the barcodes, as aggregation affects the

identification of the pathogens and the analytical performance. In contrast, when QD barcodes were stored in tablets, the fluorescence and the structure of the microspheres were maintained for up to 12 weeks at 25 and 37 °C (Figure 4a). To evaluate the diagnostic capabilities of the QD barcode tablets, we also conducted a single-plex sandwich assay to monitor the effect of the barcode's storage temperature on the analytical sensitivity of the assay (Figure 4b), or the assay's ability to detect a change in concentration.³⁰ At each time point, we added the barcode tablet (with 10–30 s dissolution) to a fluorescent DNA detection probe and solutions of known concentrations of target DNA (0–200 fmol) for 30 min and then used a magnetic field to purify the barcodes. We show that barcodes were able to consistently detect and differentiate between different concentrations of the target sequence when they were stored in tablets (Figure 4b) at 25 and 37 °C ($p = 0.0008$ and 0.0006 , respectively). In contrast, by 4 weeks at 25 °C and by 2 weeks at 37 °C, the nontableted barcodes in solution could not differentiate between different concentrations of the target DNA (Figure 4b, 4 weeks, $p = 0.206$). The polystyrene copolymer of the barcodes is held together by noncovalent forces that can be easily disrupted by temperature, resulting in surface morphological changes, agglomeration, and eventually complete degradation of the microspheres.³¹ Tablets can circumvent this by encapsulating lyophilized reagents and by minimizing contact with solvents such as water that may accelerate this degradation process. Lyophilization removes water from the microspheres' surrounding environment; limiting molecular mobility and, consequently, QD barcode degradation (Figure S4, Supporting Information).¹⁶ Encapsulation in trehalose limits the interaction of the reagents with residual water and protects the microspheres from ice formation during the freezing stage. According to the water replacement theory, trehalose hydrogen bonds with the microbeads and acts as a substitute for water; thus maintaining their structure. Other theories postulate that trehalose forms a glassy matrix to limit molecular mobility and degradation. The high glass transition temperature (T_g) of trehalose also allows the lyophilized samples to be stored at elevated temperatures.¹⁹ Finally, our compressed method can increase the densification of the powder blends, which can potentially reduce contact of the reagents with water vapor—protecting the quantum dot fluorescence from the ambient environment.³²

Application of the Tablets with Other Diagnostic Systems. To show that our tableting approach can be applied to other diagnostic assays, we evaluated tableting reagents of protein-based and genetic tests. First, we chose an enzyme-based immunoassay detecting C–X–C motif chemokine ligand 5 (CXCL5), a chemokine implicated in prostate cancer,³³ as a model protein-based diagnostic test. CXCL5 is detected when a color change occurs as a result of avidin–horseradish peroxidase (avidin–HRP) binding to an immobilized antibody–CXCL5-biotinylated antibody complex. To demonstrate the applicability of using tablets for antibodies and enzymes, we encapsulated avidin–HRP in compressed tablets. We then examined the stability of avidin–HRP in tablets for a period of 4 weeks at 25 °C. At each time point, we created a standard curve by adding different concentrations of CXCL5 to a plate of immobilized and biotinylated antibodies. Avidin–HRP tablets were then added to create a color change. At each time point, avidin–HRP remained stable and functional in compressed tablets for up to 4 weeks (Figure 5a; time 0, $p < 0.0001$; time 2, $p = 0.0005$; time 4, $p = 0.0023$). Next, we chose a nucleic acid amplification technique, called recombinase polymerase amplification (RPA), as a model

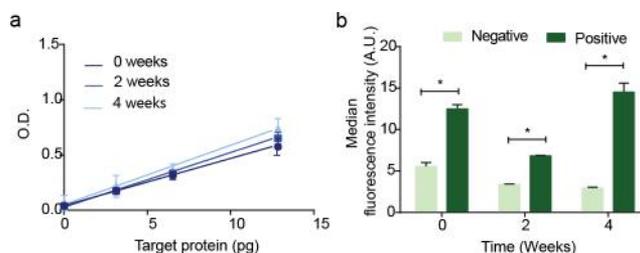


Figure 5. Characterizing reagent tablets for a protein and a genetic test. (a) Time-based standard curves conducted for an immunoassay with avidin–horseradish peroxidase (HRP) compressed tablets stored at 25 °C for 4 weeks. At each time point, avidin–HRP tablets were used to detect CXCL5 chemokine in a sandwich immunoassay format. The optical density was measured using a plate reader. Statistical significance was determined with regression analysis using GraphPad Prism software. (b) Time-based amplification of synthetic DNA using recombinase polymerase amplification tablets stored at 25 °C for 4 weeks. At each time point, RPA tablets were used to amplify water (negative control) and 10^7 copies of synthetic DNA. A sandwich assay was then conducted with barcodes coated with capture probes complementary to the amplicons and a detection probe. The results were analyzed using flow cytometry. Statistical significance was calculated using a Mann–Whitney test using SPSS software.

genetic test. This technique uses recombinase proteins, which form a nucleoprotein complex with primers, to facilitate strand exchange at homologous sequences of the template DNA. As single-stranded binding (SSB) proteins stabilize this complex, a DNA polymerase extends the template of interest to provide exponential amplification.³⁴ To demonstrate the applicability of using tablets for DNA and proteins, we encapsulated primers (Table S3, Supporting Information) and all protein components required for RPA, including recombinase, polymerase, single-stranded binding proteins, and other cofactors, in a compressed tablet. We then stored the tablets at 25 °C for 4 weeks. At each time point, the stability of RPA components in tablets was evaluated by amplifying 10^7 synthetic copies of DNA (Table S3). Overall, RPA proteins and primers remained stable at room temperature for up to 4 weeks in tablets (Figure 5b, 4 weeks, $p = 0.05$). We can therefore easily adapt this tableting technology for other diagnostic platforms without adversely affecting biomolecular reagents over time.

Clinical Testing of HBV with Multiple Tablets. Next, we evaluated whether our tableting technology can be used to simplify a multistep assay for screening patient samples. We developed three tablets: an RPA tablet, a QD barcode tablet, and a tablet containing a fluorescent reporter probe (DNA probe with Alexa Fluor 647) for clinical HBV samples (Tables S1 and S4, Supporting Information). We collected samples from hepatitis B-positive (HBV+) patients and used a combination of the genetic test and the barcode technology to demonstrate genetic detection of healthy and HBV+ patients. We first extracted nucleic acids from serum samples. We then added the RPA tablet for amplification, denatured the sample at 100 °C, and added the QD barcode tablet (containing barcodes for the HBV target) as well as the reporter probe tablet for the HBV target (Figure 6a). We screened three healthy and three HBV patient samples using all three reagent tablets and analyzed the results with a benchtop flow cytometer and a point-of-care smartphone device (Figure 6b). With the use of reagent tablets, we were able to differentiate between healthy and HBV patient samples using benchtop and point-of-care instrumentation (Figure 6c,d, $p < 0.0001$). We were therefore able to demonstrate that our tableting strategy can (a)

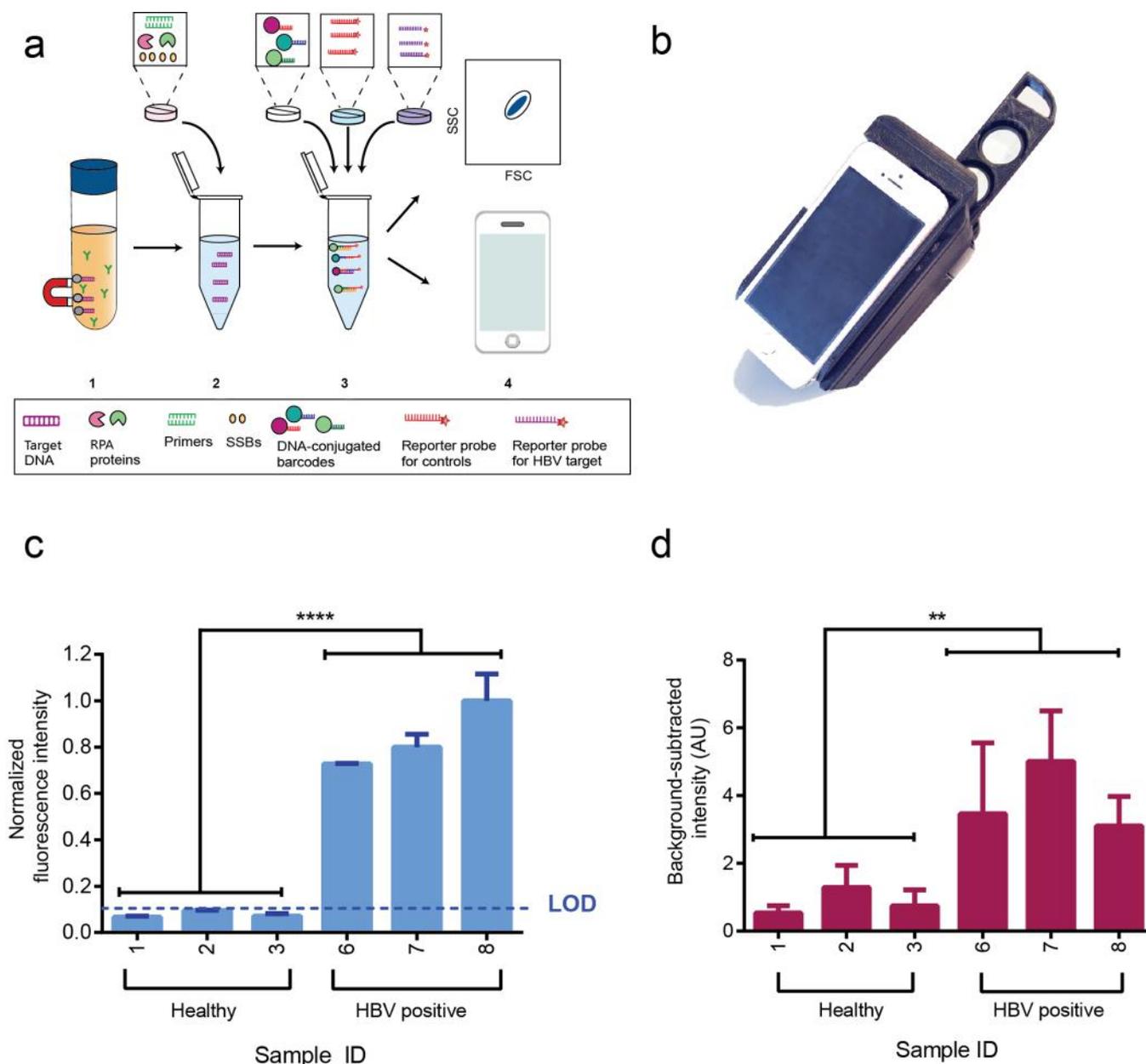


Figure 6. Screening for healthy and Hepatitis B Virus positive (HBV+) patient samples using reagent tablets in a multistep assay. (a) Schematic representation of the workflow for validation. (b) Photograph of the wireless diagnostic device. (c) Results of screening healthy and HBV+ clinical samples with a benchtop flow cytometer (viral loads of the samples can be found in Table S4, Supporting Information). Compressed tablets encapsulated with diagnostic reagents were able to differentiate between healthy and HBV+ patient samples ($p < 0.0001$) at benchtop. (d) Results of screening healthy and HBV+ clinical samples with smartphone imaging using a wireless diagnostic device. Compressed tablets encapsulated with diagnostic reagents were able to differentiate between healthy and HBV+ patient samples ($p < 0.0001$) at point-of-care. Statistical significance was determined by conducting a Mann–Whitney test between pooled healthy and HBV+ signal intensities in IBM SPSS software. LOD = limit of detection. Key: **, $p < 0.01$; ****, $p < 0.0001$.

simplify multistep diagnostic assays to (b) screen for patient samples.

DISCUSSION

In this study, we stabilized and simplified multistep diagnostic assays by using a high-throughput compression strategy to develop reagent tablets. Reagent tablets offer an inexpensive (Table S5, Supporting Information) means for providing thermal stability to reagents. This technology allows areas with high burden of diseases but low infrastructural capabilities to also have access to medical diagnostics. Reagents can degrade during

shipping and storage in tropical environments. This can compromise test results and ultimately lead to misdiagnoses and poor patient outcomes. We were able to stabilize diagnostic reagents at elevated temperatures using our tableting technology. To develop tablets, we first lyophilized reagents with trehalose. Trehalose acted as a cryoprotectant, shielding the constituents from ice crystals.¹⁹ Due to the protective properties of trehalose, a variety of reagents, ranging from antibodies to polymer microspheres, were able to be lyophilized without degradation. Following lyophilization, we encapsulated diagnostic reagents in tablets without altering the structural and functional properties of

the reagents. We were able to store polymer microspheres at elevated temperatures for 3–6 times longer than when stored in solution. We were also able to extend this technology to other types of assays which have antibodies, enzymes, and DNA, without adversely affecting the biomolecules. Furthermore, once dissolved, the tablets did not interfere with the diagnostic assays conducted. It is therefore possible to easily apply this technology and formulation to a myriad of other molecular and chemical diagnostic assays, such as protein and nucleic acid tests. In addition to providing thermal stability, reagent tablets are also easy-to-handle and provide premeasured quantities of reagents without the need for additional packaging consumables. As a result, we were able to reduce user intervention when conducting multistep assays. We screened healthy and HBV+ clinical samples by adding a series of tablets and used both benchtop and point-of-care smartphone technology for detection. Reagent tablets can therefore be used to screen clinical specimens in advanced as well as point-of-care facilities.

Compressed tablets offer a variety of advantages for pharmaceutical drug encapsulation, including providing stability, cost-effectiveness, and fast dissolution times.¹³ In this study, we were able to exploit these advantages and apply this platform to address barriers limiting the translation of medical diagnostic assays. Although it is possible to simply lyophilize the molecular reagents, their subsequent encapsulation in tablets provides an easy and simple way of developing prepackaged reagents. In the absence of prepackaged tablets, lyophilized reagents need to be resuspended, aliquoted, stored, and then measured before use, increasing the risk of introducing human error and interoperator variability. Others have demonstrated packaging lyophilized reagents in consumables,³⁵ such as Eppendorf tubes. However, this increases the cost of the assay and limits their translation to resource-poor settings (~\$20 for 100 tubes vs \$0.16 for 100 tablets). Although other groups have also demonstrated the development of reagent tablets, these were proof-of-concept studies that did not show the clinical readiness of the platform.^{11,12} To expedite the clinical translation of these technologies, it is important to demonstrate their performance in complex biological matrixes.³⁶ We have demonstrated the suitability of translating our tableting technology to resource-limited settings by showing the ease of using tablets, in combination with a smartphone camera, for screening clinical samples. Prior to using tableted reagents, the workflow for a diagnostic assay required a number of experts ranging from phlebotomists and technicians to clinicians. The lack of availability of such experts, especially in remote locations, has hindered the progression of diagnostics in resource-poor areas. State-of-the-art diagnostics therefore have not made the jump from the bench to the clinic. By introducing reagent tablets, we have simplified the workflow such that a lay person can still perform complex diagnostics with everyday household items, such as with a cup of water to dissolve the tablets or a stove to heat reactions.

While we demonstrate the utility of tableting reagents for medical diagnostic applications, we can extend this technology for detecting contaminants in the environment (lakes and rivers) or foodborne pathogens in produce (meats, dairy). We envision mixing a reagent tablet with a sample of contaminated water or a tissue sample to identify contaminants and pathogens. We expect to improve the accessibility of assays by simplifying complex reagent-based sensors. In the future, we expect the end user to add a sequence of color-coded tablets to the sample of interest, then to wait for a period of time, and finally to visualize the result

by eye by a hand-held smartphone camera. Ultimately, we envision bridging the gap between the development of diagnostic tests and their subsequent translation to resource-poor areas.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b07055.

Additional experimental details, characterization, primers and sequences, and cost analysis (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*warren.chan@utoronto.ca

ORCID

Warren C. W. Chan: 0000-0001-5435-4785

Notes

The authors declare no competing financial interest.

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