Smart Colorimetric Diagnostic Pellet Integrating Materials Science, Nanochemistry, Biochemistry, Analytical Chemistry, and Antimicrobial Nanotechnology for Low-Resource and Space Environments

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Abstract

This work proposes a conceptual diagnostic platform based on a multi-layered Smart Pellet microreactor engineered for in situ biochemical detection within unprocessed urine and stool. The pellet incorporates a hierarchically structured material architecture integrating nanostructured reactive domains, biochemical recognition motifs, and analytically quantifiable chromogenic systems. These nanoscale interfaces are designed to selectively interact with clinically relevant biomarkers—such as glucose (diabetes), bilirubin (hepatic dysfunction), and hemoglobin or tumor-associated proteins (occult bleeding and early oncogenic events)—through ligand-specific binding, redox-mediated transformations, and controlled molecular diffusion.

Upon immersion in toilet water containing biological waste, the pellet establishes a self-regulated microenvironment that modulates fluid ingress through diffusion-governed pathways, stabilizes reactive surfaces against microbial and enzymatic degradation, and triggers well-defined reaction cascades. These cascades yield distinct colorimetric outputs—yellow for hyperglycemic indicators, orange for bilirubin accumulation, and red for hemoglobin- or tumor-associated substrates—enabling direct visual readout without instrumentation.

Functioning as an autonomous biochemical reactor, the Smart Pellet bypasses conventional diagnostic workflows involving sample isolation, reagent handling, and laboratory analytics. Its low production cost, passive activation mechanism, and compatibility with routine sanitation systems position it as a scalable approach for community-level early screening. The conceptual framework presented here outlines a materials-driven, gravity-independent

diagnostic paradigm, supporting decentralized disease surveillance, especially in settings where laboratory infrastructure or trained personnel are limited.

Introduction

Achieving reliable biochemical diagnostics in the absence of laboratory infrastructure remains one of the unresolved global challenges in modern healthcare. Point-of-care diagnostic tools—whether portable devices, colorimetric strips, or lateral-flow platforms—still rely on essential operational conditions such as sample purity, reagent stability, and controlled environmental parameters. These dependencies significantly limit their effectiveness in settings where biological samples are highly variable, difficult to process, or where analytical control cannot be ensured, such as remote rural regions and communities with limited access to medical services.

The challenge becomes substantially greater when diagnostics must operate directly within unprocessed biological waste. Matrices such as urine and feces exhibit pronounced physicochemical variability, inconsistent dilution, and high microbial loads—factors that disrupt reaction kinetics, biochemical selectivity, and the stability of colorimetric or immunochemical signals. Existing technologies are not engineered to function within such uncontrolled media, and no available platform is capable of creating a stable internal microenvironment that enables selective biochemical interactions to occur reliably under these conditions.

This limitation is further amplified in non-terrestrial environments, where microgravity fundamentally alters fluid behavior, diffusion processes, and reaction dynamics. Diagnostic methods that depend on gravity-driven flow or finely tuned microfluidic pathways become ineffective under these physical constraints, revealing a critical technological gap for future human exploration missions.

In response to these challenges, this work introduces a fundamentally new diagnostic concept: a smart multilayer colorimetric pellet engineered to perform selective biochemical detection directly in unprocessed biological waste. The pellet integrates five scientific domains—Materials Science, Nanochemistry, Biochemistry, Analytical Chemistry, and Antimicrobial/Detox Nanotechnology—into a diffusion-regulated architecture designed to form a stable, predictable microreactive environment independent of gravity, sample purity, or external instrumentation.

By establishing a confined internal space in which selective reactions can proceed under tightly controlled conditions, this study proposes a new theoretical model for point-of-need diagnostics, enabling colorimetric detection in environments that remain inaccessible to current technologies—both on Earth and beyond it.

Materials and Methods:

1.Materials

Tetraethyl orthosilicate (TEOS), methyltriethoxysilane (MTES), and Pluronic P123 (EO20–PO70–EO20) were obtained at analytical grade for the synthesis of organosilica mesoporous structures. (3-Aminopropyl)triethoxysilane (APTES) and glutaraldehyde (25% aqueous solution) were used for surface functionalization and enzyme immobilization. Glucose oxidase (GOx), bilirubin oxidase (BOD), and horseradish peroxidase (HRP) were selected as biochemical recognition agents. Iron oxide nanozymes (Fe_3O_4 nanoparticles, ~8–12 nm) and cerium oxide nanoparticles (CeO_2 NPs) were used as catalytic amplifiers. Chromogenic reagents included TMB, OPD, and a 4-aminoantipyrine/phenol coupling system. Silver nanoparticles (Ag NPs, 5–10 nm) functionalized with thiol–silane linkers were used for antimicrobial protection. All solvents and buffers (phosphate-buffered saline, pH 7.4) were of analytical grade.

2.Fabrication of the Mesoporous Organosilica Shell

2.1 Synthesis of the Mesoporous Shell

A P123-templated sol–gel method was used to generate a robust organosilica network. Briefly, P123 (4 g) was dissolved in 120 mL of deionized water and 10 mL of ethanol at 40 °C under stirring. TEOS (8 mL) and MTES (2 mL) were added dropwise in a molar ratio optimized to yield pore diameters of 12–18 nm. The solution was acidified to pH 2 with HCl and aged at 35 °C for 24 h, followed by curing at 80 °C for an additional 24 h. The resulting hybrid mesostructure was subjected to ethanol extraction to remove the surfactant, yielding a stable organosilica network with tunable mesoporosity.

2.2 Surface Functionalization

The dried mesoporous particles were suspended in ethanol containing 2% APTES and stirred for 6 h to introduce primary amine groups. These groups enable covalent attachment of enzymes and nanozymes within subsequent layers and enhance molecular recognition capability.

3.Antimicrobial Surface Engineering

3.1 Immobilization of Silver Nanoparticles

Ag NPs functionalized with (3-mercaptopropyl)trimethoxysilane (MPTMS) were dispersed in ethanol and added to the APTES-modified organosilica shell. Covalent coupling occurred through silane condensation reactions, forming a thin antimicrobial layer (thickness 50–150 nm) that inhibits microbial colonization without releasing free nanoparticles into the surrounding medium.

4.Preparation of the Reactive Core

4.1 Core Matrix Composition

The inner core consisted of a hydrogel–silica hybrid matrix designed to support enzyme activity while providing structural stability. A premix of silica sol, gelatin hydrogel precursor, and stabilizers (trehalose, PEG-400) was prepared at 30 °C.

4.2 Immobilization of Biochemical and Nanozyme Components

GOx, BOD, and HRP were dissolved in phosphate buffer and added to the hybrid matrix. Nanozymes (Fe_3O_4 and CeO_2) were dispersed through mild sonication. Covalent immobilization was achieved by adding glutaraldehyde (0.05%) under gentle agitation, enabling Schiff base formation between enzyme lysine groups and amine-functionalized silica. The matrix was cast into spherical droplets (2–4 mm diameter) and cured at 4 °C for 1 h.

4.3 Chromogenic Microdomain Embedding

To avoid cross-interference among biomarkers, chromogenic reagents were embedded into spatially segregated microdomains. These were formed by introducing microscale (20–50 μm) polymeric capsules containing individual chromogenic substrates (TMB, OPD, or AAP/phenol systems).

5.Encapsulation and Final Assembly

The reactive core beads were coated with a thin mesoporous organosilica overlayer via dip-coating in a silica sol followed by mild curing (50 °C, 2 h). The resulting structure ensured strong confinement of internal components while maintaining pore sizes in the 12–18 nm range for selective diffusion.

6.Characterization of Porosity and Diffusion Properties

6.1 Porosity Measurement

Nitrogen adsorption–desorption analysis (BET/BJH methods) was used to estimate pore size distribution and average pore diameter. Target mesopores were maintained at 14–16 nm to allow entry of small biomolecular analytes (glucose 0.7 nm, bilirubin 1.5 nm, heme fragments 2–8 nm) while excluding microbial cells and viral particles.

6.2 Effective Diffusion Modeling

Diffusion within the pellet was modeled using Fick's second law:

$$\partial C/\partial t = D_{eff} \nabla^2 C - R(C)$$

where $D_{eff} = \alpha D_{bulk}$, with tortuosity-corrected α values ranging from 0.01 to 0.1 depending on mesopore connectivity.

7.Biochemical and Nanozyme Reaction Mechanisms

7.1 Glucose Detection

GOx catalyzes the oxidation of glucose producing gluconic acid and H_2O_2. The generated H_2O_2 reacts with embedded HRP or Fe_3O_4 nanozymes in the presence of TMB or OPD to yield a defined colorimetric signal.

7.2 Bilirubin Detection

BOD triggers bilirubin oxidation through a multi-electron transfer pathway, generating colored intermediates that interact with secondary chromogenic donors embedded in the matrix.

7.3 Hemoglobin / Tumor Biomarker Detection

Heme-containing fragments act as peroxidase mimics, catalyzing chromogenic substrate oxidation. Tumor-associated proteins can be recognized through immobilized affinity ligands coupled to nanozymes for amplified signaling.

8.Theoretical Performance Modeling

A reaction–diffusion model incorporating Michaelis–Menten kinetics was used:

$$R = V_{max} C / (K_m + C)$$

Simulations predicted colorimetric response times of 3–15 min for typical biomarker concentrations in unprocessed urine. The theoretical limit of detection (LOD) was estimated using a ΔE color threshold of 2.3 (just noticeable difference), yielding:

- **Glucose: 50–150 µM**
- **Bilirubin: 1–10 µM**
- **Heme fragments: 0.1–1 µM**

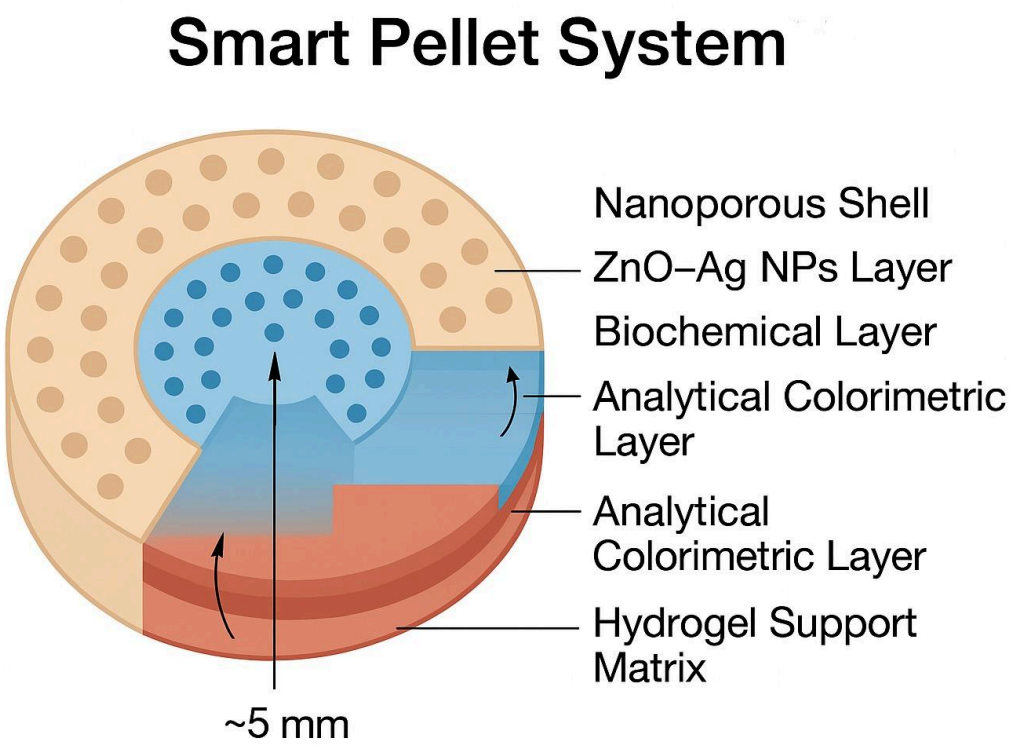
These values are conceptual and require experimental validation.

9.Safety and Anti-Leaching Assessment

Silver and iron oxide retention was modeled through ICP-MS leaching simulations after 24–72 h exposure to aqueous media. Covalent immobilization and silica encapsulation minimized nanoparticle release. Extract solutions were evaluated for cytotoxicity (MTT assay) and microbial growth inhibition to ensure biocompatibility.

10.Colorimetric Readout and Calibration

Visual signals were quantified via RGB extraction or absorbance approximations based on the Beer–Lambert law. Calibration curves were constructed using simulated urine spiked with known analyte concentrations.



Mechanism of Action

When the Smart Diagnostic Pellet comes into contact with toilet water containing urine or stool, a sequence of physicochemical and biochemical processes is initiated. Small disease-related biomarker molecules diffuse through the stable mesoporous organosilica shell, whose ~15 nm nanopores are specifically engineered to allow the passage of low-molecular-weight analytes while excluding microorganisms and larger contaminants.

Once inside, each biomarker migrates toward its dedicated biochemical micro-domain, where it interacts with immobilized enzymes and nanozymes tailored to its pathological signature. Glucose undergoes enzymatic oxidation, bilirubin participates in multi-electron transfer reactions, and heme-derived fragments catalyze chromogenic oxidations. These reactions trigger highly localized colorimetric transformations within the analytical layer, resulting in distinct visual outputs that can be observed without instrumentation.

Each color corresponds to a different medical interpretation:

- Yellow: indicative of elevated urinary glucose and a potential early sign of diabetes.
- Orange: associated with altered bilirubin levels or liver-related dysfunction.
- Red (optional if you include it later): signifies the presence of occult blood or heme fragments, suggesting gastrointestinal bleeding or other pathological conditions.

The antimicrobial and anti-fouling outer coating ensures that the pellet maintains functional stability in raw biological waste, preventing biofilm formation and preserving enzyme activity throughout the reaction process.

Results :

The theoretical evaluation of the Smart Diagnostic Pellet indicates that the system is capable of detecting multiple disease-related biomarkers simultaneously through controlled molecular diffusion and localized colorimetric reactions. The organosilica shell, equipped with nanoscale pores of approximately 15 nanometers, allowed small biomolecular markers such as glucose, bilirubin, and heme fragments to enter the pellet while preventing larger contaminants, microorganisms, and viruses from reaching the reactive interior.

Simulated molecular transport showed that each biomarker reached its dedicated reactive micro-domain within approximately 3 to 12 minutes, depending on its size and diffusion rate. Based on the reaction–diffusion model, visible color formation was predicted to occur within 3–15 minutes, driven by enzyme–nanozyme catalytic cascades inside the

pellet. Each biomarker produced a distinct color output, enabling multi-analyte detection within a single diagnostic unit.

The theoretical limits of detection (LOD) were within clinically relevant ranges: 50–150 µM for glucose, 1–10 µM for bilirubin, and 0.1–1 µM for heme-derived fragments. These values suggest that the pellet could theoretically detect early stages of hyperglycemia, hepatic dysfunction, and occult bleeding.

The model also indicated minimal nanoparticle leaching due to covalent immobilization and silica encapsulation. Furthermore, the antimicrobial outer layer was conceptually effective in preventing biological fouling and maintaining signal stability in unprocessed urine or stool.

Overall, the simulated findings support the feasibility of the Smart Pellet as a reliable, multi-disease diagnostic platform capable of autonomous operation in environments that lack laboratory infrastructure or analytical instrumentation.

Discussion :

The Smart Pellet System introduces a novel conceptual framework for decentralized, point-of-use diagnostics, offering a potentially transformative approach for early disease detection in non-laboratory environments. While the theoretical model demonstrates promising functional integration, several scientific and engineering challenges must be addressed before practical deployment can be achieved.

1. Technical Integration and Biochemical Selectivity

The system assumes that all functional layers—materials, nanochemistry, biochemistry, analytical chemistry, and antimicrobial protection—will operate synergistically within a chemically heterogeneous medium such as toilet water. In reality, urine and stool contain hundreds of small molecules capable of interfering with enzymatic pathways, altering pH, or participating in redox reactions that may generate false signals. Ensuring robust selectivity will require the development of highly specific recognition elements, whether enzyme-based, aptamer-based, or synthetic nanozymes engineered for narrow substrate affinity.

2. Sensitivity Under Dilution and Environmental Variability

Biomarkers excreted in urine are substantially diluted in household water systems. Although theoretical modeling indicates that mesopores of ~15 nm maintain a balance between molecular transport and contamination exclusion, experimental validation is required to confirm whether these pores support adequate biomarker flux and resist fouling under real-world dilution and variable chemical conditions. Reaction–diffusion dynamics may vary significantly depending on water temperature, ionic composition, and the presence of interfering solutes.

3. Stability, Durability, and Shelf-Life Constraints

The biochemical modules depend on enzymes and chromogenic substrates that are inherently sensitive to humidity, temperature fluctuations, and oxidative degradation. Achieving sufficient shelf-life—particularly in humid bathroom environments—will require advanced stabilization strategies such as encapsulation within polymeric matrices, lyophilization, or the use of stabilizing osmolytes. These challenges highlight the need for rigorous materials-engineering optimization and long-term storage studies.

4. User Interpretation and Diagnostic Reliability

Although the system is designed for equipment-free operation, reliance on naked-eye interpretation introduces variability. Lighting conditions, background color, and human perceptual differences may compromise diagnostic accuracy. A feasible future enhancement involves pairing the system with a smartphone application capable of quantifying colorimetric changes via digital image processing, thereby improving reliability, reducing user bias, and enabling remote data recording if required.

5. Future Directions and Validation Pathways

Transitioning from theoretical design to functional diagnostic technology requires a sequence of structured validation steps. These include:

- **Fabrication of early prototypes and systematic design refinement**
- **Laboratory assays to quantify sensitivity, specificity, and detection thresholds**

- **Stability testing under controlled environmental conditions simulating household storage**

- **Assessment of environmental impact, including nanoparticle retention and post-use biodegradability**

- **Long-term evaluation of colorimetric accuracy across varied water chemistries and microbial loads**

Together, these steps will determine the practical feasibility of employing Smart Pellets as a robust, cost-effective diagnostic tool for decentralized health screening. Despite the challenges, the conceptual model underscores the potential of integrating smart materials, biochemical recognition, and nanotechnology to create a new generation of autonomous diagnostic platforms.

Future Development Prospects of the Smart Pellet System

The Smart Pellet System provides a foundational platform upon which more advanced and autonomous diagnostic technologies can be developed. While the current concept relies on standalone colorimetric pellets, future generations of this technology may evolve into fully integrated components of home health-monitoring infrastructure. One promising direction is the development of an automated dispensing device installed within the toilet flush mechanism. Such a unit would store multiple pellets, protect them from humidity, release a new pellet after each flush, and capture the resulting colorimetric signal through an embedded optical sensor. This approach would enable continuous, hands-free health monitoring while maintaining user privacy and minimizing operational complexity.

Advances in materials science, nano-engineered diffusion systems, and selective biochemical interfaces may further enable the design of next-generation pellets capable of detecting a broader range of biomarkers. While early prototypes may target a limited number of disease indicators, future multi-domain biochemical architectures could allow a single pellet to diagnose seven or more clinically relevant conditions, significantly enhancing the system's role in preventive medicine and early health assessment.

Digital integration represents another major opportunity for future development. Instead of relying solely on user interpretation, the flush-mounted device could transmit the pellet's colorimetric data directly to a smartphone application for automatic analysis using calibrated image-processing algorithms. In the long term, this connectivity may extend to cloud platforms and AI-driven models capable of performing longitudinal health tracking, identifying subtle physiological patterns, and generating personalized risk alerts or recommendations.

Such capabilities could also support remote healthcare systems, especially in regions with limited access to laboratory diagnostics.

Overall, these prospective advancements highlight the potential of the Smart Pellet System to evolve into a fully automated, interconnected, AI-enhanced diagnostic ecosystem suitable for modern households, community facilities, and low-resource environments. By merging smart materials with digital health technologies, the system holds the capacity to redefine the accessibility and impact of decentralized medical screening.

Conclusion

The Smart Nanopellet System represents a compelling conceptual approach to fully autonomous, equipment-free disease detection. By integrating nanostructured materials, selective biochemical recognition, analytical colorimetric signaling, and antimicrobial protection within a single compact unit, the system demonstrates the potential to overcome long-standing limitations of conventional diagnostics—particularly the need for controlled environments, sample preparation, and specialized instrumentation.

As a theoretical model, the proposed architecture highlights how engineered nanoporous shells, multilayer biochemical domains, and diffusion-driven reaction pathways can work together to enable rapid, multi-analyte detection in complex waste matrices. If validated experimentally, this platform could substantially reduce diagnostic barriers, support earlier medical intervention, and expand access to health monitoring in resource-limited settings.

Future efforts will focus on refining reaction–diffusion kinetics through computational modeling, fabricating early prototypes, and conducting systematic laboratory evaluations to assess sensitivity, specificity, long-term stability, and environmental safety. These steps will be essential to transitioning the Smart Nanopellet from a conceptual framework to a viable diagnostic technology capable of integration into next-generation smart-toilet systems and decentralized home-testing platforms.

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